Category A

OH&RM ONE HEALTH & RISK MANAGEMENT

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The Moldovan Association for Biosafety and Biosecurity (MDBBA) is a scientific and practical, instructive and educational, non-governmental, apolitical and non-profit professional organization, created in 2017.

The main objective of the association is the development of good practices and culture in the field of biosafety and biosecurity and the promotion of knowledge within professional and research-innovation groups.

Biosafety – includes security principles, technologies and rules to be followed to prevent unintended exposure to pathogens and toxins or their accidental release/leakage.

"Protection of personnel, population from unintended exposure to pathogens/biohazardous material".

Biosecurity - includes a wide spectrum of measures (biosecurity policies, regulatory regime, scientific and technical measures) applied in an organized framework, necessary to minimize risks (prevention of actions, terrorist attacks by the intentional release of pathogens or toxins as well as loss, their theft or misuse).

"Protection and prevention of theft, intentional misuse of pathologies/biohazardous material".

Risk management – is a decision-making process in which the results of risk assessment (the process of estimating workplace hazards) are integrated with economic, technical, social and political principles to generate strategies for risk reduction.

OH&RM ONE HEALTH &
RISK MANAGEMENT

CONTENTS –TABLE DES MATIÈRES

FOREWORD – AVANT-PROPOS

Gheorghe SAVUȚA, prof. univ., dr., Directorul Şcolii Doctorale de Medicină Veterinară, Universitatea de Științele Vieții, Iași, România

Împreună, spre „O singură sănătate" !

SYNTHESIS ARTICLES – ARTICLES DE SYNTHÈSE

RESEARCH ARTICLES – ARTICLES DE RECHERCHE

Împreună, spre *O singură sănătate* !

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Cercetarea științifică și inovarea sunt activități acceptate și recunoscute la nivel mondial și au un rol esențial în dezvoltarea economică și în progresul social al unei țări, reprezentând principalul factor de creștere a productivității muncii, a eficienței utilizării resurselor materiale și energetice, precum și a competitivității economiei pe termen lung. Cercetătorii se străduiesc în mod constant să exploreze noi frontiere și să facă descoperiri inovatoare. O importanță incontestabilă o au platformele dedicate utilizării rezultatelor științifice pe diferite domenii. Schimbul de informații între cercetători impulsionează creșterea performanțelor științifice și contribuie la o permanentă competiție pentru atingerea obiectivelor, oferind soluții la provocările actuale. Revista „One Health & Risk Management" este o platformă de conectare a cercetătorilor din cele trei domenii științifice cheie ale abordării conceptului „O singură sănătate". Este confirmat faptul că sănătatea oamenilor și cea a animalelor sunt interdependente și legate de ecosistemele în care coexistă și evoluează constant. Conform datelor Organizației Mondiale a Sănătății, peste 70% dintre bolile contagioase ale omului provin din contactul cu animalele precum și din consumul de produse de origine animală contaminate cu germeni patogeni zoonotici. Există deasemenea o serie de agenți chimici și radioactivi, proveniți din activitatea omului prin poluare și contaminare, ceea ce influențează schimbările climatice, iar ca urmare, oamenii și animalele suportă consecințele. Vorbind despre conceptul „O singură sănătate", se subînțelege necesitatea ca cercetătorii din diverse domenii să-și unească eforturile pentru îmbunătățirea sănătății globale, prin comunicare, coordonare și colaborare științifică. În lumea interconectată de azi, persoanele și mărfurile se deplasează pe distanțe mari, iar politicile implementate într-o regiune pot avea un impact important în altă regiune. În acest context, soluționarea problemelor existente impune implicarea sporită a cercetării științifice și consolidarea colaborării dintre organizațiile responsabile de aceste domenii: Organizația Mondială a Sănătății, Organizația Națiunilor Unite pentru Alimentație și Agricultură și Organizația Mondială pentru Sănătatea Animalelor. În acest context, revista științifică „One Health & Risk Management" vine cu un aport major de consolidare a domeniilor și tematicilor noi de cercetare științifică cu referire la sănătatea umană și animală, precum și la protecția mediului.

SYNTHESIS ARTICLE – ARTICLES DE SYNTHÈSE

MEDICAL SCIENCES

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SEXUAL AND REPRODUCTIVE HEALTH IN MEN WITH SPINAL CORD INJURY

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Introduction. Sexual health is a fundamental aspect of overall well-being, quality of life, and a sense of fulfillment. A significant percentage of men with spinal cord injury (SCI) experience ejaculatory issues (95%), poor semen quality (75%), and erectile dysfunction (80%), making it impossible for them to conceive without medical assistance.

Material and methods. The bibliographic resources were analyzed and selected from databases such as PubMed, SCOPUS, HINARI, and Web of Science to identify studies on the desired topic. Following the initial research, 128 items were identified, and 32 representative articles were selected for this synthesis article.

Results. Damage to the spinal cord can result in partial or complete loss of function below the level of the injury. These injuries lead to impaired ejaculatory and erectile function in addition to reproductive issues, which might include abnormal sperm motility, morphology, and viability, as well as reduced spermatogenesis. Challenges such as loss of bladder and bowel function, changes in physical appearance, spasms, lack of communication, and stereotypes can affect sexual life of men with SCI.

Conclusions. Studying sexual dysfunction in disabled men is essential for promoting comprehensive healthcare, supporting mental health, fostering healthy relationships, and advocating for the rights and inclusion of individuals with disabilities. Understanding the mechanisms affecting sexual function in people with SCI is of particular practical interest both in medical practice and in subsequent studies on patients.

Cuvinte-cheie: leziunea măduvei spinării, disfuncție sexuală, fertilitate, dizabilitate fizică, sănătate reproductivă.

MECANISMELE DISFUNCȚIILOR SEXUALE LA BĂRBAȚII CU LEZIUNI ALE MĂDUVEI SPINĂRII

Introducere. Sănătatea sexuală este un aspect fundamental al bunăstării generale, calității vieții și sentimentului de împlinire. Un procent semnificativ de bărbați cu leziuni ale măduvei spinării (LMS) se confruntă cu probleme ejaculatorii (95%), calitate slabă a spermei (75%) și disfuncție erectilă (80%), făcând imposibilă conceperea fără asistență medicală.

Material și metode. Resursele bibliografice au fost analizate și selectate din baze de date precum PubMed, SCOPUS, HINARI și Web of Science pentru a identifica studii privind subiectul vizat. În urma cercetării inițiale au fost identificate 128 de articole, dintre care 32 de articole reprezentative au fost selectate pentru acest articol de sinteză.

Rezultate. Leziunile la nivelul măduvei spinării pot duce la pierderea parțială sau completă a funcțiilor situate sub nivelul leziunii. Aceste leziuni duc la afectarea funcției ejaculatorii și erectile, precum și la probleme de reproducere, care pot include motilitatea, morfologia și viabilitatea anormală a spermatozoizilor, inclusiv reducerea spermatogenezei. Provocări precum pierderea funcției vezicii urinare și a funcției intestinale, modificări ale aspectului fizic, spasme, lipsa de comunicare și stereotipuri pot afecta viața sexuală a bărbaților cu LMS.

Concluzii. Studierea disfuncției sexuale la bărbații cu dizabilități este esențială pentru promovarea asistenței medicale complete, susținerea sănătății mintale, încurajarea relațiilor sănătoase și susținerea drepturilor și incluziunii persoanelor cu dizabilități. Definirea mecanismelor care afectează funcția sexuală la persoanele cu LMS prezintă un interes deosebit atât în practica medicală, cât și în studiile ulterioare asupra pacienților.

INTRODUCTION

Sexual health is a fundamental aspect of overall well-being, quality of life, and a sense of fulfillment. The World Health Organization declared everyone's right to sexual health to be fundamental in 2000. They defined sexual health as "a state of physical, emotional, mental, and social well-being in relation to sexuality; it is not merely the absence of disease, dysfunction, or infirmity" (1).

Men with physical disabilities encounter a unique set of challenges that can impact various aspects of their lives, including intimate relationships and personal well-being. Understanding the mechanisms and effects of physical disabilities is vital for advancing medical knowledge and developing effective treatments. By studying physical disabilities, researchers can work towards reducing social stigma and promoting inclusion. A deeper understanding of these conditions helps challenge stereotypes, fostering a more inclusive and supportive attitude towards individuals with physical disabilities.

There is a common but inaccurate assumption that people with disabilities may not be interested in or capable of engaging in sexual activities. This misconception contributes to the lack of emphasis on studying sexual dysfunction within this population. An estimated 1.3 billion people experience significant disability. This represents 16% of the world's population. Nearly 200 million of them experience difficulties in functioning (2). Traumatic spinal cord injury (SCI) most commonly occurs in young males, at the peak of their reproductive health (3). A significant percentage of men with SCI experience ejaculatory issues (95%), poor semen quality (75%), and erectile dysfunction (80%), making it impossible for them to conceive without medical assistance (4).

The *aim* of this study was to define the morphological and pathophysiological mechanisms, clinical symptoms, psychological changes, and management of sexual dysfunction in men with SCI.

MATERIAL AND METHODS

The bibliographic resources were selected from databases such as *PubMed*, *SCOPUS*, *HINARI*, and *Web of Science* using keywords such as "spinal cord injury," "sexual dysfunction," and "reproductive health," which were used in various combinations to maximize search efficiency.

For targeted selection of bibliographic sources, the following filters were applied: full-text articles, articles in the English language, and articles related to male sex. A total of 128 articles matching the search criteria were found. After excluding records unrelated to the study's aim, 32 representative articles, including different types of publications such as WHO documents, books, reviews, clinical studies, and sociological studies, were considered potentially relevant for the given synthesis.

Articles that did not align with the purpose of the study and were not available for full viewing, articles with insufficient information, articles lacking data on sexual dysfunction in men with SCI, and studies on pediatric populations (<17 years) were excluded from the bibliography.

RESULTS

Spinal cord injury (SCI) is a condition that results in either temporary or permanent changes to the cord's function. There are two categories of SCI aetiologies: traumatic and non-traumatic. Non-traumatic SCI occurs when an acute or chronic disease, such as a tumor, infection, or degenerative disc disease, causes the primary injury. Traumatic SCI often results from accidents such as car crashes, falls, or sports injuries. Damage to the spinal cord can result in partial or complete loss of function below the level of the injury (5), as the injury can be complete or incomplete (fig. 1).

Central Cord Syndrome

Sensory and motor deficits occur below the injury level. The upper extremities are affected more than the lower extremities because the axons of the corticospinal tracts that supply the upper extremities are situated closer to the spinal cord, while those that supply the lower extremities are located closer to the periphery. The mechanism of injury is hyperextension, and it is frequently observed in elderly patients (7).

Anterior Cord Syndrome

Bilateral loss of motor function, pain, and temperature sensation below the injury level (injury to the spinothalamic tracts). Intact vibration and proprioception (unaffected dorsal columns).

Posterior Cord Syndrome

Loss of vibratory, proprioceptive, and tactile sen-

ses (injury to the dorsal columns). Intact pain, temperature sensation, and motor function (unaf-

fected spinothalamic and corticospinal tracts).
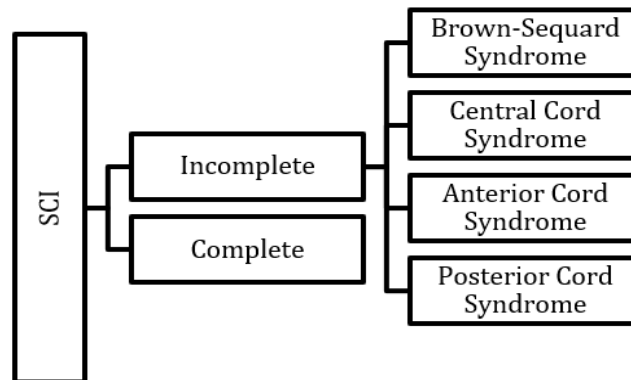
Figure 1. Spinal cord injury classification.

Complete Transection of the Spinal Cord
Complete loss of motor function on both sides, as well as proprioception, tactile, temperature, vibratory, and pain perception below the injurylevel (8).

The clinical outcomes of SCI depend on the severity and location of the lesion (tab. 1) (9). Studies have shown that individuals with tetraplegia die at a higher rate (80%) than those with paraplegia (20%), most likely as a result of respiratory compromise (10).

Table 1. Spinal cord injury levels and their effects.

Region	Subregion	Injury impact on body's functions
CERVICAL	**C1-C4**	Quadriplegia May not be able to breathe on their own. Requires complete assistance with activities.
	C5-C6	Paralysis in wrists, hands, trunk, and legs. Can raise their arms, bend elbows, and speak but breathing will be weakened.
	C7-C8	Paralysis in hands, trunk, and legs. Has some hand movement. May be able to grasp and release objects. Horner's syndrome (miosis, ptosis, and facial hypohidrosis) may accompany a cervical cord lesion at any level.
THORACIC	**T1-T5**	Paralysis in trunk and legs. Have normal arm, hand, and upper-body movement. Disturbances of bladder and bowel function.
	T6-T12	Paralysis in trunk and legs. Affects the abdominal, lower back muscles, and the legs.
LUMBAR	**L1-L5**	Some loss of function in the hips and legs. Little or no voluntary control of the bowel or bladder. Sexual function may be affected.
SACRAL	**S1-S5**	Prominent bladder and bowel dysfunction (urinary retention and incontinence with lax anal tone), and impotence. The bulbocavernosus (S2-S4) and anal (S4-S5) reflexes are absent.

Mechanism of Neurogenic Shock

The primary causes of spinal cord injury are disruptions to the neural parenchyma and axonal networks of the spinal cord caused by damage to astrocytes, neurons, microglia, and oligodendrocytes (11). Secondary injury results from swelling, inflammation, reactive oxygen species, and

the release of excitatory amino acids that inhibit neuronal repair and growth.

Due to sympathetic blunting in SCI above T6, the somatic and autonomic systems experience an acute state of neurogenic shock, which includes areflexia, profound unopposed parasympathetic dominance, resulting in neurogenic bradycardia,

neurogenic orthostatic hypotension (NOH), bronchiolar constriction, mucus secretion, and priapism in addition to the sensory and motor paralysis associated with traumatic SCI. Furthermore, hypovolemic shock related to blood loss from internal injuries is common in patients with traumatic SCI; this condition should be accompanied by compensatory tachycardia, but the parasympathetic dominance prevents this from happening. To maintain the proper cardiac output in cases of acute neurogenic bradycardia linked to high thoracic and cervical SCI, transcutaneous pacing may be necessary (12). The immediate result of SCI is vascular damage, which is followed by ischemia. Vasospasm, intravascular thrombosis, and primary disruption of vessels ultimately lead to local ischemia. Due to its higher metabolic demands, the highly vascularized grey matter containing neurons is more vulnerable to injury. When an injury is severe enough, there is a noticeable disruption of the grey matter as the injury volume increases and blood is extravasated (13).

Erectile Dysfunction

There are two main types of erection:
1. Reflexogenic erection (S2-S4 nerve roots intact) is caused by direct stimulation of the penis. The spinal parasympathetic reflex pathway located in the sacral spinal cord is involved. The sacral spinal cord receives sensory data from the penis upon physical contact, which causes motor output and penile erection. Reflex erections can still occur in SCI patients if the sacral spinal cord is unharmed.
2. Psychogenic erection (T11-L2 nerve roots intact). It has been demonstrated that the sympathetic nervous system regulates psychogenic erections, which are induced by ideas, images, sounds, and fantasies rather than genital stimulation. The penis becomes erect as a result of signals sent by supraspinal centers to the sympathetic preganglionic neurons in the spinal cord following stimulation. SCI at or above T11 disrupts supraspinal control and decreases psychogenic erections (14).

Erection occurs when the formation of nitric oxide (NO) increases by the non-adrenergic/non-cholinergic neurons and endothelial cells, which stimulates the formation of cyclic guanosine monophosphate (cGMP), resulting in a de creesed level of intracellular calcium. This decrease causes the smooth muscle to relax, leading to an erection. Phosphodiesterase type 5 (PDE5) breaks down cGMP to return it to the flaccid state. The inhibition of PDE5 results in the maintenance of high levels of cGMP, and therefore, the maintenance of the erection (15).

Anejaculation

Ejaculation depends on the proper functioning of the parasympathetic, sympathetic, and somatic nervous systems, in addition to an intact ejaculatory reflex.

There are two phases of ejaculation:
1. *Emission* - Seminal fluids and sperm deposit in the posterior urethra, then the seminal vesicles and prostate contract. This phase is mediated by the sympathetic nervous system (the ejaculation center is between T12 and L2).
2. *Expulsion* - The ejection of semen through the urethral meatus. The bladder neck closes (preventing retrograde ejaculation), the pelvic floor and bulbocavernosus muscles contract (S2-S4), and then the external urinary sphincter relaxes. This phase is mediated not only by the sympathetic nervous system but also by the parasympathetic and somatic nervous systems (15).

The integration between spinal centers and the autonomic nervous system is organized by interneurons that shape the spinal ejaculation generator (SEG), which is responsible for ejaculation. The location of the SEG in male humans has recently been determined to be in the L3–L5 segment (16).

Semen Quality

Males with SCI often have variable morphology, low sperm motility and viability, and altered semen plasma content. Though controversial, the root cause of low sperm quality in men with SCI is complex and may involve anejaculation, infections of the reproductive tract, prostatic dysfunction, and/or an inability to control scrotal temperature as a result of wheelchair use. Other factors that may adversely affect fertility include changes to structural proteins, mitochondrial activity, and DNA damage (17).

Most men with SCI cannot ejaculate by masturbation, so they require medical assistance for sperm retrieval (electroejaculation, vibratory stimulation).

Moreover, according to a study, 27% of men produced brown-colored semen (the cause may be related to seminal-vesicle dysfunction), but this was not associated with the duration of anejaculation or frequency of ejaculation. Additionally, other changes were present, such as leukocytospermia (not associated with genitourinary tract infection), and low viability and motility of sperm (15).

Consistent with earlier reports, one study found no relationship between the time after SCI and the main sperm parameters, including semen volume, total sperm count, progressive motility, vitality, and normal sperm morphology (18).

Genetic and Epigenetic Involvement

In recent years, the modern literature has offered few genetic studies on infertile males with SCI. Indeed, there are no genome-wide association studies (GWAS). In contrast to cancer, which is related to heritable somatic genes that can be studied, it is important to consider that the lack of GWAS is caused by the isolated nature of the event. Nonetheless, a genetic reason could explain the ways in which SCI affects germline DNA and why the manifestation and severity of infertility in men with SCI vary widely. It is well known that DNA methylation and histone modification impact spermatogenesis, fertilization, and early embryogenesis.

According to one study on rats, the critical neuron population that makes up the spinal ejaculation generator experienced a long-term decrease in galanin and GRP mRNA following contusion injury at mid-thoracic spinal levels. A critical future question to be addressed is identifying the mechanisms by which SCI causes a reduction in neuropeptide expression in lumbar spinothalamic cells. These modifications seem to be the consequence of long-term changes in supraspinal influences and epigenetic modifications leading to reduced mRNA expression of the neuropeptides rather than acute inflammatory processes occurring at the site of injury (19).

Psychological changes

Numerous studies have examined quality of life indicators related to sexual activity in men with SCI. Between 30-70% of men with SCI are sexually inactive (20, 21). Various challenges can affect sexual life, such as changes in physical appearance, loss of bladder and bowel function, spasms, erectile dysfunction, lack of communica-

tion, weakness, low sexual desire, and stereotypes (22).

People with disabilities encounter social norms that primarily assume them to be asexual, immature, and having low sexual desire (23). For both the patient and their family, a spinal cord injury is a very stressful and overwhelming condition. A significant component of the clinical care of patients with this condition must be patient education. Counseling is required regarding the prognosis, complications, and outcomes. Support groups can assist in managing problems such as depression, loneliness, frustration, and anxiety. Studies involving healthcare professionals have revealed that they frequently see numerous obstacles when it comes to discussing sexuality with their patients (24).

Anxiety is considered the primary cause of rapid ejaculation. The sympathetic nervous system increases anxiety levels and is responsible for rapid ejaculation, whereas low anxiety delays ejaculation (25). Cortisol is a hormone associated with mental stress and high alertness in stressful situations. A recent study showed a relationship between cortisol, stress, and premature ejaculation (26).

Many patients are uncomfortable talking about their sexuality, are unsure of which healthcare professional to ask their questions to or are unsure of the provider's comfort level when it comes to sexual health (27).

Treatment options

Oral phosphodiesterase-5 inhibitors (PDE-5 inhibitors) are commonly used to treat erectile dysfunction in men with spinal cord injuries (fig. 2). They usually respond well to this medication (3). Intracavernosal injections, surgical penile prostheses, and specialized vacuum devices are possible options for men with SCI who do not respond well to PDE-5 inhibitors. Considering that most of them cannot ejaculate, techniques like penile vibratory stimulation (PVS) or electroejaculation (EEJ) are highly successful in obtaining sperm for artificial insemination.

Surgical sperm retrieval is regarded as the last option when EEJ and PVS are not successful or tolerated. Current advances in assisted reproductive technologies, such as intravaginal insemination, intrauterine insemination, and in vitro fertilization, have led to favorable pregnancy rates despi-

te impairments in semen quality in men with spinal cord injuries (28).

The process of adjustment can be aided by behavior modification and the treatment of depression and anxiety using methods like cognitive behavioral therapy, mindfulness-based cognitive therapy, interpersonal psychotherapy, and psychoeducation (29).
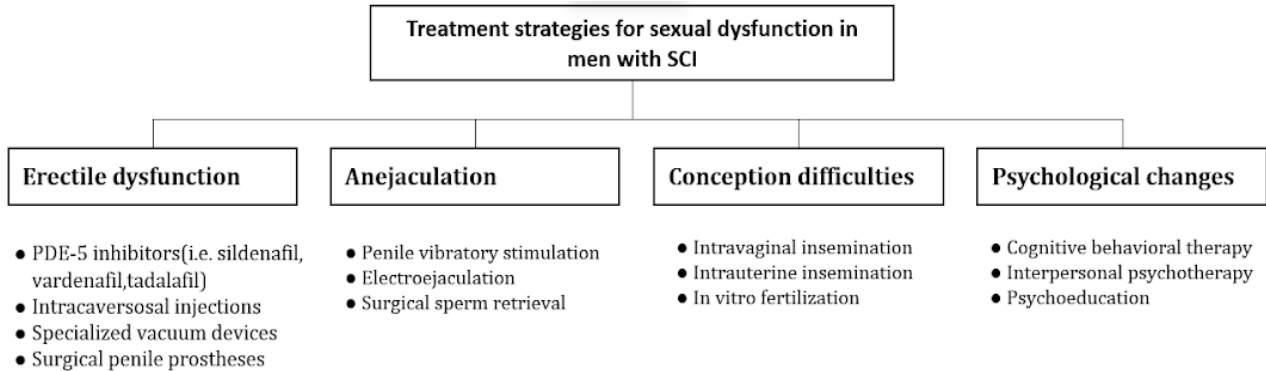
Figure 2. Sexual dysfunction treatment options in men with SCI.

DISCUSSIONS

Most men with SCI have severe impairments in their reproductive and sexual functioning as a result of abnormalities in their semen and/or ejaculatory, erectile dysfunction (30). In addition to the direct and indirect effects of neuronal damage in people with spinal cord injury, social, cultural, and personal contexts may also affect an individual's capacity to view sexual activities as pleasurable and fulfilling (31). There is substantial evidence to support the idea that societal stigmas and misconceptions about people with disabilities, which see them as asexual, create obstacles to men with traumatic spinal cord injuries receiving sexual health rehabilitation (32).

It's important to note that while genetic studies may provide valuable insights into the mechanisms underlying infertility in men with SCI, comprehensive clinical assessments and interdisciplinary approaches involving urologists, endocrinologists, and reproductive specialists are typically necessary to evaluate and manage infertility in this population.

The reviewed articles predominantly agree that men with traumatic spinal cord injuries have medical options to address their sexual health. Treatment should be integrated with the patient's residual sexual potential. Considering the complex nature of sexual dysfunction and its effects on multiple aspects of patients' quality of life, pharmacological and psychological approaches may be combined to produce appropriate therapy alternatives.

Comprehensive, long-term studies are crucial in understanding the evolving nature of sexual dysfunction in men with SCI and the effectiveness of various interventions over time. It is also essential to develop and implement training programs for healthcare providers to enhance their knowledge and sensitivity in addressing the sexual health concerns of men with SCI.

CONCLUSIONS

1. SCI remains an important cause of sexual dysfunction in men, affecting each aspect of their lives. Studying sexual dysfunction in disabled men is essential for promoting comprehensive healthcare, supporting mental health, fostering healthy relationships, and advocating for the rights and inclusion of individuals with disabilities. It contributes to a clearer understanding of the challenges faced by this population and facilitates the development of targeted interventions and support systems.

2. Current studies in the field of sexual health of people with physical disabilities are limited, with the vast majority approaching the subject from a social rather than a medical perspective. Understanding the mechanisms affecting sexual function in people with SCI is of particular practical interest, both in medical practice and in subsequent studies on patients.

3. A wealth of evidence indicates that stigma from society and oneself maintains stereotypes about men with disabilities as asexual and/or unattractive, which adds even more obstacles to the provision of sexual health rehabilitation services for men who have suffered traumatic spinal cord injuries.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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MEDICAL SCIENCES

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EVOLUTION OF INFLUENZA AND ACUTE RESPIRATORY INFECTIONS IN THE REPUBLIC OF MOLDOVA DURING THE 2014/15 TO 2022/23 EPIDEMIC SEASONS

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Keywords: influenza, acute respiratory infections, severe acute respiratory infections.

Introduction. *Influenza-like illness (ILI), Acute Respiratory Infections (ARI), and Severe Acute Respiratory Infections (SARI) are the most common infectious diseases globally, causing severe forms annually, especially in at-risk groups. The aim of this paper is to analyse the epidemiological and virological data on ILI, ARI, and SARI in the Republic of Moldova during the 2014/2015 to 2022/2023 epidemic seasons.*

Material and methods. *The descriptive-retrospective epidemiological study analysed morbidity due to ILI, ARI, and SARI, as well as laboratory results for the presence of influenza viruses during the 2014/2015 to 2022/2023 epidemic seasons.*

Results. *ILI has been of low to medium intensity except for two seasons, while ARI has been of very high intensity in one season. ILI, ARI, and SARI primarily affected children aged 0-14 years. During the nine seasons (except 2020/2021), influenza virus types A(H1N1) pdm09, A(H3N2), and B have been identified, with the percentage of positives decreasing considerably in the last two seasons. During the period under review, each influenza virus type dominated, and in three seasons, two to three virus types co-dominated. Genetic characterization of the identified strains demonstrated that influenza viruses fully fit the phylogenetic tree constructed for the nominated period, do not differ antigenically from strains identified in other regions of the northern hemisphere, and are susceptible to antivirals.*

Conclusions. *The national surveillance system for ILI, ARI, and SARI makes it possible to monitor the evolution of the epidemic process in real time and geographic space, facilitating prompt intervention with control and response measures.*

Cuvinte-cheie: gripa, infecții acute ale căilor respiratorii superioare, infecții respiratorii acute severe.

EVOLUȚIA GRIPEI ȘI A INFECȚIILOR RESPIRATORII ACUTE ÎN REPUBLICA MOLDOVA ÎN PERIOADA SEZOANELOR 2014/2015-2022/2023

Introducere. *Gripa, infecțiile acute ale căilor respiratorii superioare (IACRS) și infecțiile respiratorii acute severe (SARI) sunt cele mai răspândite boli infecțioase la nivel global, provocând anual forme severe în special la grupele de risc. Scopul lucrării este de a analiza datele epidemiologice și virologice privind gripa, IACRS și SARI în Republica Moldova, în perioada sezoanelor 2014/2015 – 2022/2023.*

Material și metode. *În studiul epidemiologic descriptiv-retrospectiv a fost analizată morbiditatea prin gripă, IACRS și SARI și rezultatele de laborator la prezența virusurilor gripali în perioada sezoanelor 2014/2015-2022/2023.*

Rezultate. *Gripa a avut o intensitate de nivel jos și mediu cu excepția a 2 sezoane, iar IACRS a înregistrat într-un sezon un nivel de intensitate foarte înaltă. Gripa, IACRS și SARI a afectat primordial copii de 0-14 ani. Pe parcursul celor 9 sezoane (excepție 2020/2021) au fost identificate tipurile de virusuri gripale: A(H1N1) pdm09, A(H3N2) și B, procentul pozitivității reducându-se considerabil în ultimele 2 sezoane. În perioada analizată a dominat unul dintre virusurile gripale, iar în 3 sezoane au dominat concomitent 2-3 tipuri de virusuri. Profilul genetic al tulpinilor identificate a demonstrat că virusurile gripale se încadrează totalmente în arborele filogenetic construit în perioada nominalizată, nediferențiindu-se antigenic de tulpinile identificate în alte regiuni ale emisferei de nord și fiind sensibile la antivirale.*

Concluzii. *Sistemul național de supraveghere a gripei, IACRS și SARI face posibilă monitorizarea eficientă a evoluției procesului epidemic în timp real și spațiu geografic, favorizând o intervenție promptă cu măsuri de control și răspuns.*

INTRODUCTION

Influenza-like illness (ILI), as well as acute and severe viral respiratory infections, are among the most widespread infectious diseases worldwide, accounting for up to 95% of infectious diseases in some countries (in the Republic of Moldova), they account for up to 89.7% of infectious diseases). Due to the level of morbidity and mortality they cause, these infections have a negative impact on the health of the population, the health system, and the national economy. Experts estimate that the costs associated with acute respiratory infections worldwide amount to about $25 billion (1).

Seasonal influenza viruses are continuously evolving and cause severe illness every year, especially in older adults, children, pregnant women, and individuals with chronic health issues. From 2002 to 2011, approximately 389,000 respiratory deaths linked to influenza occurred annually worldwide (uncertainty range of 294,000 to 518,000) (2–5). Among these, persons aged 65 and above accounted for 67% (2, 3). The World Health Organization (WHO) in 2019 declared influenza and potential pandemic influenza strains as one of the most important threats to global health (6).

WHO has implemented the Global Influenza Strategy (2019-2030) to strengthen country capacity and preparedness for future pandemics by improving seasonal influenza prevention, surveillance, and control (7). Enhancing surveillance, monitoring, and utilization of global influenza data, alongside promoting research and innovation, are two core objectives of this strategy.

Currently, about 200 antigenically distinct viruses from 10 genera are known to cause acute respiratory illness, but the most dangerous, the most studied, and, at the same time, the most unknown and unpredictable remain the influenza viruses (1).

In this context, WHO recommends that all National Influenza Centers conduct surveillance for ILI, acute respiratory infections (ARI), and severe acute respiratory infections (SARI) according to geographical spread, intensity and trend of the epidemic process, epidemic threshold, dominant/co-dominant influenza virus strains, antiviral resistance, and influence on the health system in order to reduce the socio-economic impact caused by morbidity and mortality from these infections and improve surveillance and respons measures (8).

The aim of this paper is to analyze the epidemiological and virological situation of ILI, ARI, and SARI morbidity in the Republic of Moldova during the 2014/2015 to 2022/2023 epidemic seasons.

MATERIAL AND METHODS

A descriptive analysis of the annual evolution of epidemiological and virological data for ILI, ARI, and SARI was performed. Data collection was carried out through the national surveillance and monitoring system of ILI, ARI, and SARI in all administrative territories of the Republic of Moldova according to Annex No. 2 (Reporting form on cases of ILI, ARI, and SARI in the district) of the Ministry of Health Order No. 792/2023 (11,063 annexes) (9). The epidemic threshold for ILI and ARI, as well as the medium, high, and very high thresholds, were calculated by ECDC and WHO specialists based on data uploaded weekly to the European surveillance portal for infectious diseases (EpiPulse) by the National Influenza Centre of Moldova.

Detection of influenza viruses in nasopharyngeal exudate samples from individuals with a presumeptive diagnosis of influenza, ARI, or SARI was performed using molecular biology techniques (rRT-PCR).

Isolation of influenza viruses was performed in MDCK and MDCK-SIAT1 cell cultures according to WHO-recommended methodology. Identification of isolated strains was performed by hemagglutination-inhibition assay with reference antisera to influenza A(H1N1) pdm09, A(H3N2), and B viruses. Genetic characterization of influenza virus strains was performed using molecular biology techniques (PCR-real time, sequencing), and the susceptibility of isolated strains to anti-influenza remedies (oseltamivir, zanamivir) was determined by the neuraminidase inhibition test, following the method recommended by the World Health Organization in collaboration with the Francis Crick National Institute for Health Research in London, UK.

Statistical data processing was performed using Microsoft Excel 365 and Epi Info 7.2. Descriptive statistics, including incidence rates and proportions, were calculated for the variables of interest. The data were presented as percentages, accompanied by 95% confidence intervals (CI) for proportions.

RESULTS

During the 2014/2015 to 2022/2023 epidemic seasons, a total of 13,074 cases of ILI were registered, of which 75.9±0.7% were hospitalized. The highest percentage (24.9±0.7%) of the total number of registered ILI cases was in the 2019/2020 season, followed by the 2018/2019 season with 16.8±0.6%, the 2014/2015 season with 15.3±0.6%, and the 2022/2023 season with 15.1±0.6%. In the 2020/2021 season, no influenza cases were recorded.

The calculation of the ILI epidemic threshold started in the 2018/2019 season, initially being 2.09%$_{000}$ cases of ILI, and then increased to 5.72%$_{000}$ cases from the 2020/2021 season. Analyzing the evolution of ILI morbidity in these seasons (fig. 1), it is highlighted that the 2019/2020 season is the only season that exceeded the mid-level epidemic threshold for ILI (14.22%$_{000}$) during weeks 08/2020 to 09/2020, reaching the highest incidence in the last nine seasons of 16.5%$_{000}$ ILI cases.
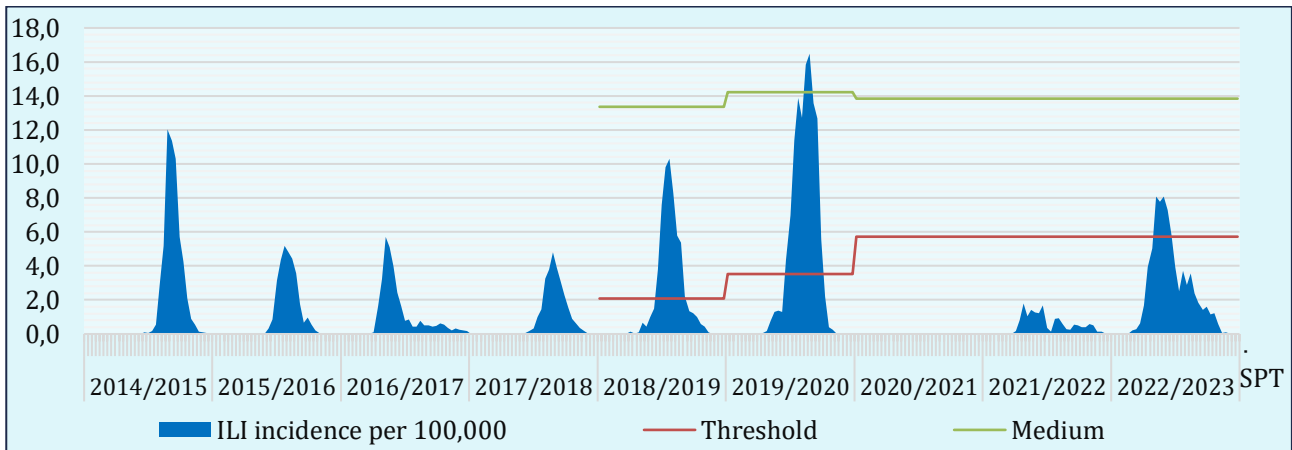
Figure 1. Evolution of ILI morbidity during the 2014/2015 to 2022/2023 epidemic seasons, per 100,000 population.

Only in two seasons (2016/2017 and 2022/2023), the maximum weekly incidence was recorded at the beginning of December in week 51 of 2017 and 2023, with 5.1%$_{000}$ and 8.1%$_{000}$ ILI cases respectively. In two seasons (2020/2021 and 2021/2022), the evolution of ILI remained below the epidemic threshold. In the other seasons, the maximum weekly incidence was recorded during weeks 06-09 (February).

In five of the seasons analyzed, ILI predominantly affected children in the 0-14 age group, ranging from 54.4±3.1% (2017/2018) to 64.4±4.5% (2021/2022). The 2014/2015 season was the only one in which the 30+ age group had a proportion of 53.0±2.2% (fig. 2). The fewest cases were recorded in the 65+ age group, ranging from 3.3±0.7% in the 2019/2020 season to 8.9±1.3% in the 2014/2015 season.
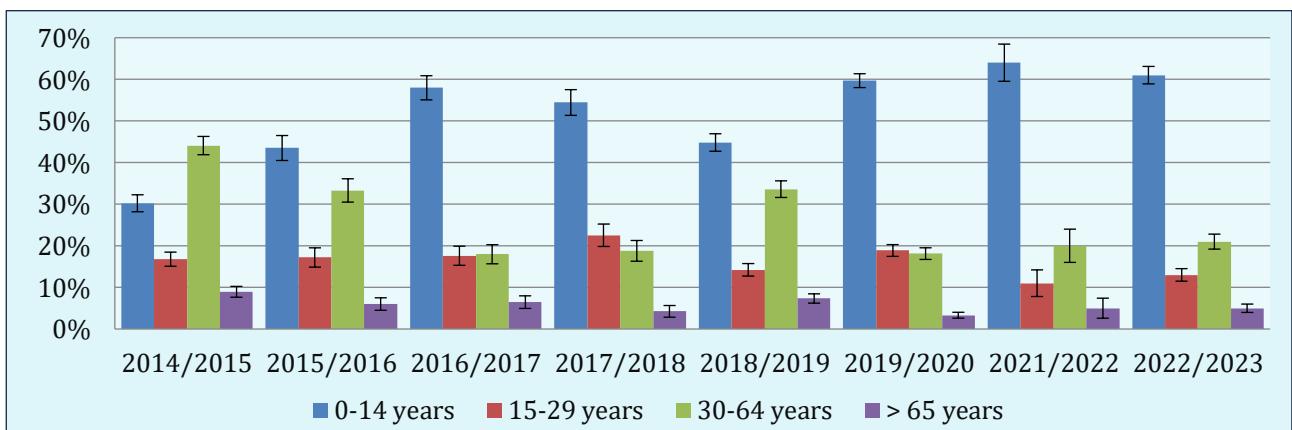
Figure 2. Percentage of ILI cases by age, 2014/2015 to 2022/2023 epidemic seasons.

In total, 2,120,942 cases of ARI were recorded in the RM over nine seasons, ranging from 6.8±0.03% (2020/2021) to 13.7±0.04% (2018/2019) of the total per season. Of the total number of cases, only 3.7±0.03% were hospitalized. Acute respiratory infections were recorded in all administrative territories.

As in the case of ILI, the maximum weekly incidence of ARI in two seasons was recorded in early December, specifically in week 51 of 2017 and 2023, with 447.5%$_{ooo}$ and 636.3%$_{ooo}$ cases respectively. In the 2020/2021 season, the evolution of ARI remained below the epidemic threshold of 283.63%$_{ooo}$, and in the 2021/2022 season, the maximum weekly incidence was recorded in week 03 with 443.96%$_{ooo}$. In the other five seasons, the maximum weekly incidence was recorded during weeks 06-09 (February). Only in the last season, 2022/2023, a very high ARI intensity level of 636.29%$_{ooo}$ was recorded (fig. 3).
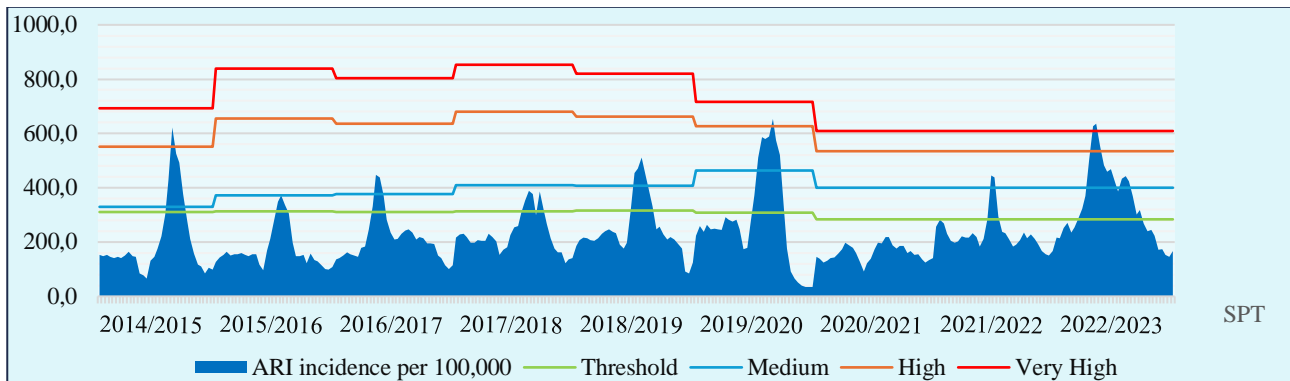
Figure 3. Evolution of ARI morbidity during the 2014/2015 to 2022/2023 epidemic seasons, per 100,000 population.

In the case of acute respiratory infections, each season saw the highest proportion of cases in children aged 0-14 years, ranging from 50.6±0.3% in the 2020/2021 season to 69.1±0.2% in the 2016/2017 season (fig. 4). In the age group 65+ years, the lowest proportion of cases was recorded, ranging from 2.6±0.1% in the 2016/2017 season to a maximum of 7.5±0.1% in the 2020/2021 season.
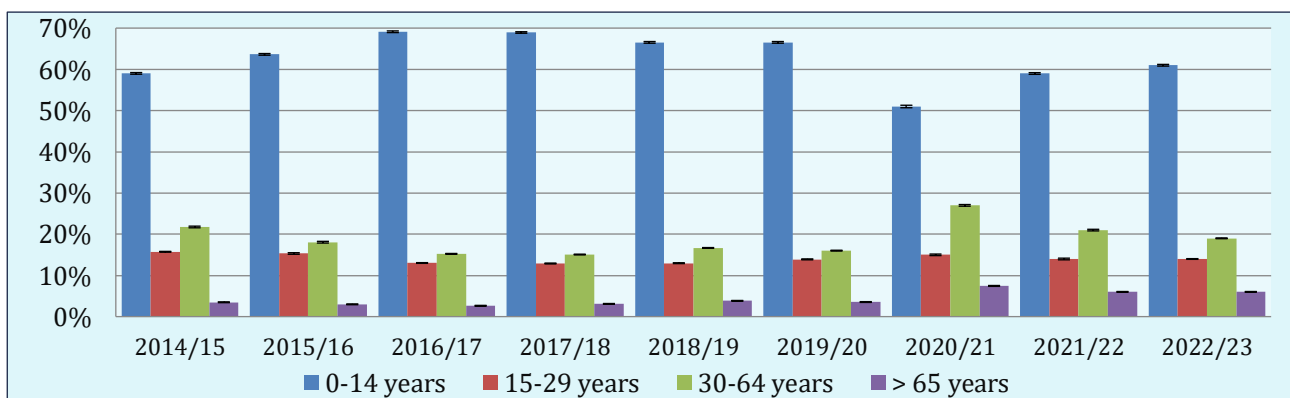
Figure 4. Percentage of ARI cases by age, 2014/2015 to 2022/2023 epidemic seasons.

A total of 157,441 cases of severe acute respiratory infections (SARI) were recorded during the 2014/2015 to 2022/2023 epidemic seasons, all of which were hospitalized according to the case definition. The highest proportion (17.9±0.2%) of all SARI cases occurred in the 2016/2017 season, followed by the 2015/2016 season with 16.8±0.2% and the 2014/2015 season with 14.4±0.2%. The lowest proportion was recorded in the 2019/2020 season with 6.2±0.1%.

In the 2020/2021 influenza season alone, the peak of 40.2%$_{ooo}$ SARI cases was reached in week 08, while the season before that had the lowest rate of all seasons, with 2.6%$_{ooo}$ SARI cases in week 18 (fig. 5).
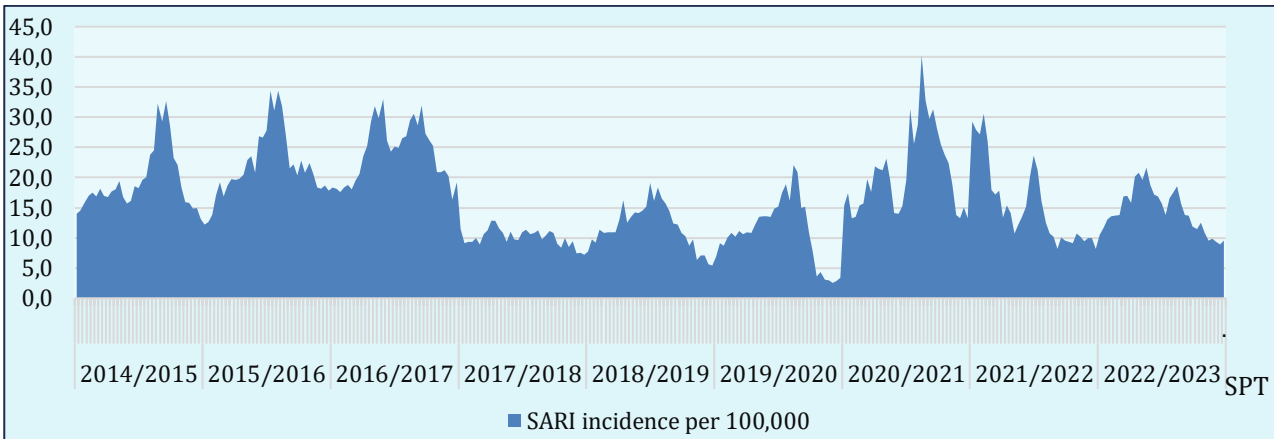
Figure 5. Evolution of SARI morbidity during 2014/2015 to 2022/2023 epidemic seasons, per 100,000 population.

The age group 0-4 years was the most affected by severe acute respiratory infections among all age groups, with a proportion ranging from 39.7±0.9% in the 2022/2023 season to 59.3±0.6% in the 2015/2016 season. Exceptions occurred in the 2020/2021 season, when the 30-64 years age group had the highest share at 49.3±0.7%, and in the 2021/2022 season, when the 30-64 years age group also had the highest share at 29.9±0.8% (fig. 6). SARI cases in the last three seasons have practically doubled in the 65+ age group compared to previous seasons, with percentages increasing from 11.6±0.4%-15.5±0.7% to 22.6±0.7%-31.8±0.7%.
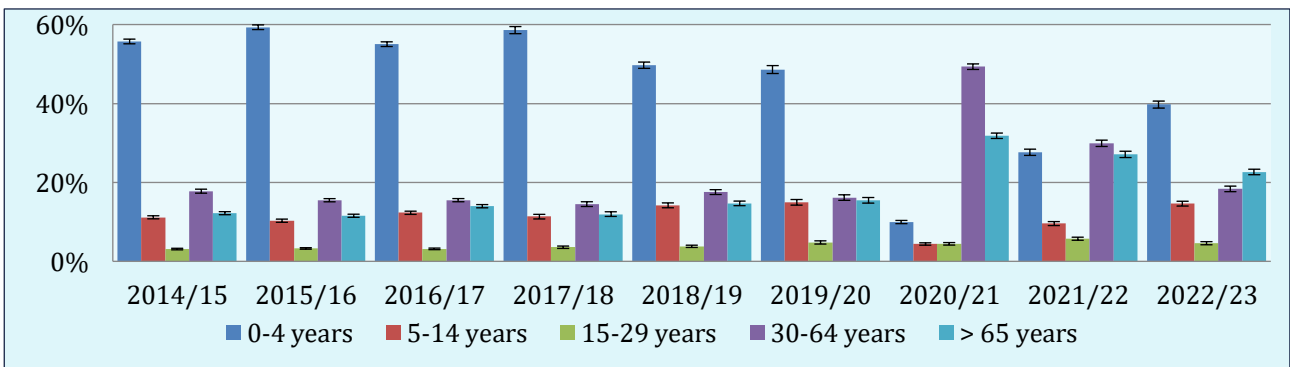
Figure 6. Percentage of SARI cases by age, 2014/2015 to 2022/2023 epidemic seasons.

During the 2014/2015 to 2022/2023 epidemic seasons, no major gender difference was observed in the registration of cases of ILI (women – 50.3±0.9%), acute respiratory infections (women – 2.5±0.07%), and severe acute respiratory infections (women – 49.0±0.2%).

During the 2014/2015 to 2022/2023 epidemic seasons, a total of 17,194 nasopharyngeal exudate samples were investigated for the presence of influenza viruses, of which 12.7±0.5% (2,178 samples) were positive for influenza viruses A(H1N1) pdm09, A(H3N2), and B. In the first six seasons analyzed, the proportion of negative results ranged from 62.5±2.7% (2018/2019 season) to 80.0±2.5% (2017/2018 season) (fig. 7).

After the 2020/2021 influenza season, when no influenza virus was detected in 682 samples, the share of negative results increased to 96.8±0.4% and 91.2±0.8% in the following seasons, respectively.

Analyzing this period in terms of dominance and co-dominance of influenza virus strains, three seasons were marked by the co-dominance of two to three virus types: the 2014/2015 season with co-dominance of A(H1N1)pdm09 (47%) and type B (48%) influenza viruses, the 2019/2020 season with co-dominance of A(H1N1)pdm09 (39%), type B (27%), and A(H3N2) (22%) influenza viruses, and the 2022/2023 season with co-dominance of A(H3N2) (35%), A(H1N1)pdm09 (31%),

and type B (30%) influenza viruses. In the 2015/2016 and 2018/2019 seasons, the A(H1N1)pdm09 influenza virus strain was dominant with 85% and 80%, respectively. In the other two seasons, the influenza A(H3N2) virus strain dominated with 62% and 64% respectively (2016/2017 and 2021/2022). The type B strain was dominant only in the 2017/2018, with 81%.
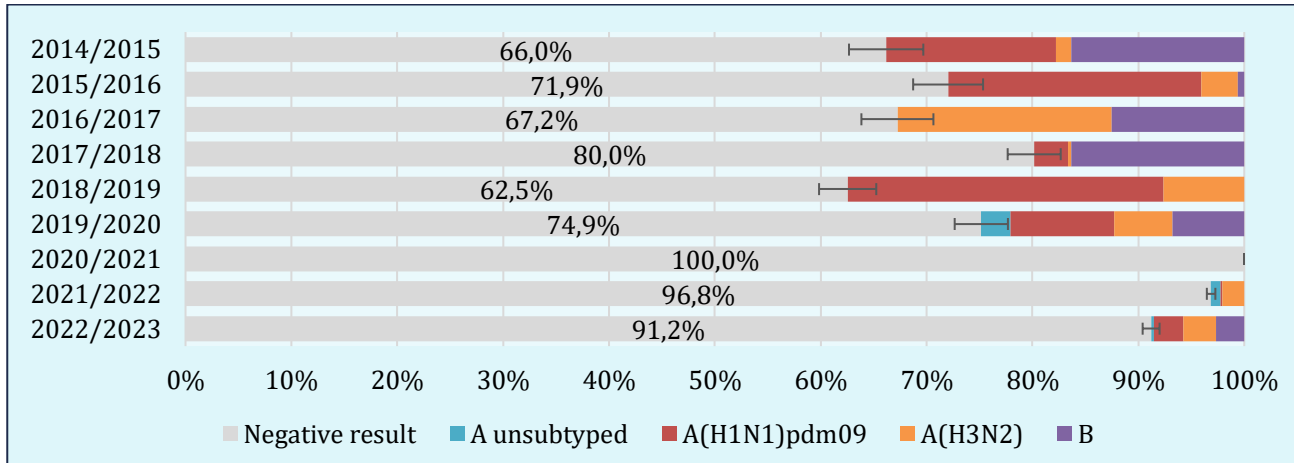
Figure 7. Percentage of influenza viruses identified during the 2014/2015 to 2022/2023 epidemic seasons.

Apart from the 2020/2021 season, the A(H1N1)pdm09 virus was not detected in the 2016/2017 season, and the type B virus was not detected in the 2018/2019 and 2021/2022 seasons.

Of the total 17,194 nasopharyngeal exudate samples investigated, 1,706 (9.9%) samples were from patients with the presumptive diagnosis "ILI," 13,215 (76.9%) samples from patients with the presumptive diagnosis "ARI," and 2,273 (13.2%) samples from patients with the presumptive diagnosis "SARI" (tab. 1).

During the 2014/2015 to 2022/2023 epidemic seasons, of the 1,706 samples from patients with the presumptive diagnosis "ILI," influenza A and B viruses were detected in 44.7%. Out of 13,215 samples of nasopharyngeal exudates from patients with the presumptive diagnosis "ARI," influenza viruses type A and B were identified in 7.4%, and out of 2,273 samples from patients with the presumptive diagnosis "SARI," these influenza viruses were detected in 19.2%.

During the 2014/2015 to 2022/2023 epidemic seasons, 176 strains of influenza viruses were isolated and identified in MDCK cell cultures and shown to fit the phylogenetic tree constructed during the nominated period. All viruses identified and subsequently tested had sufficient sialidase activity to assess resistance to oseltamivir and zanamivir inhibitors in sialidase inhibition assays. All were sensitive to both inhibitors.

DISCUSSIONS

Since the 2017/2018 season, the SARI case definition was implemented according to WHO recommendations, which caused a 2.4-fold decrease in the number of cases compared to the previous season. Although the SARI case definition was changed, the age group 0-4 years remained the most affected.

As a result of the implementation of strict public health measures during the initial year of the COVID-19 pandemic, no influenza virus was detected in the investigated samples in the 2020/2021 season in the Republic of Moldova. This phenomenon was also observed in the European Region of the World Health Organization, which recorded a 99.8% reduction in positive detections of influenza virus in the sentinel system (33 out of 25,606 tested; 0.1%) (10).

Since the last two seasons, multiplex RT-PCR tests have been included in the laboratory system. This combined test detects three types of viruses simultaneously: SARS-CoV-2, influenza A, and influenza B viruses. As a result, the number of tests has increased considerably, accounting for 77% of the total number of tests in these nine epidemic seasons.

Genetic characterization of the identified strains demonstrated that the influenza viruses fully fit into the phylogenetic tree constructed during the nominated period, do not differ antigenically

Table 1. Results of laboratory investigations of samples from patients with a presumptive clinical diagnosis of ILI, ARI, or SARI for the presence of influenza viruses during the 2014/2015 to 2022/2023 epidemic seasons.

Diagnosis	Season	No. of samples examined	Influenza viruses detected				
			A Un-sub-typed N. (% (95%CI))	A(H1N1)pdm09 N. (% (95%CI))	A(H3N2) N. (% (95%CI))	B N. (% (95%CI))	A + B N. (% (95%CI))
ILI	2014/15	98	-	22 (22.4±9.0%)	3 (3.1±5.0%)	38 (38.8±9.9%)	-
	2015/16	124	-	55 (44.4±8.8%)	5 (4.0±4.7%)	2 (1.6±3.6%)	1 (0.8±3.1%)
	2016/17	133	-	-	51 (38.3±8.5%)	21 (15.8±6.9%)	-
	2017/18	134	-	8 (6.0±5.05%)	1 (0.7±2.9%)	54 (40.3±8.5%)	-
	2018/19	400	-	199 (49.7±4.9%)	33 (8.2±3.0%)	-	-
	2019/20	349	23 (6.6±3.0%)	52 (14.9±4.0%)	25 (7.2±3.1%)	37 (10.6±3.6%)	1 (0. ±1.1%)
	2020/21	60	-	-	-	-	-
	2021/22	121	-	-	19 (15.7±7.3%)	-	-
	2022/23	287	3 (1.0±1.8%)	47 (16.4±4.6%)	25 (8.7±3.7%)	37 (12.9±4.3%)	-
ARI	2014/15	342	-	31 (9.1±3.4%)	5 (1.5±1.7%)	45 (13.2±3.9%)	-
	2015/16	291	-	38 (13.1±4.2%)	11 (3.8±2.7%)	1 (0.3±1.3%)	-
	2016/17	285	-	-	52 (18.2±4.8%)	41 (14.4±4.4%)	-
	2017/18	448	-	11 (2.5±1.8%)	2 (0.4±1.0%)	63 (14.1±3.5%)	1 (0.2±0.9%)
	2018/19	526	-	104 (19.8±3.6%)	47 (8.9±2.7%)	-	-
	2019/20	509	6 (1.2±0.6%)	29 (5.7±2.3%)	28 (5.5±2.2%)	28 (5.5±2.2%)	3 (0.6±1.0%)
	2020/21	420	-	-	-	-	-
	2021/22	6194	60 (1.0±0.3%)	9 (0.1±0.1%)	92 (1.5±0.3%)	-	8 (0.1±0.1%)
	2022/23	4200	9 (0.2±0.2%)	75 (1.8±0.4%)	102 (2.4±0.5%)	77 (1.8±0.4%)	1 (0.02±0.1%)
SARI	2014/15	242	-	56 (23.1±5.6%)	2 (0.8±1.9%)	28 (11.6±4.7%)	2 (0.8±1.9%)
	2015/16	251	-	66 (26.3±5.7%)	7 (2.8±2.6%)	1 (0.4±1.6)	-
	2016/17	269	-	-	36 (13.4±4.5%)	24 (8.9±3.9%)	-
	2017/18	297	-	9 (3.0±2.5%)	-	26 (8.8±3.6%)	1 (0.3±1.3%)
	2018/19	310	-	66 (21.3±13.8%)	14 (4.5±2.8%)	-	-
	2019/20	197	-	22 (11.2±5.0%)	5 (2.5±3.0%)	6 (3.0±3.2%)	-
	2020/21	202	-	-	-	-	-
	2021/22	284	-	1 (0.4±1.4%)	25 (8.8±3.7%)	-	-
	2022/23	221	1 (0.5±1.8%)	9 (4.1±3.3%)	17 (7.7±4.1%)	12 (5.4±3.6%)	1 (0.5±1.8%)

from strains identified in other regions of the northern hemisphere, and are sensitive to antivirals (oseltamivir and zanamivir). These phylogenetic analyses have been essential for understanding the evolution and diversity of influenza viruses. This information has also been used to guide the selection of strains included in the influenza vaccine for the northern hemisphere each influenza season.

Continuous epidemiological and virological surveillance provides crucial information for the control of the epidemiological situation at the national level and allows prompt and targeted interventions if needed. In this way, health authorities can implement public health measures adapted to

the situation, helping to limit the spread of infections and protect public health.

Currently, the health system of the Republic of Moldova has a routine and sentinel surveillance system for ILI, ARI, and SARI, adjusted to WHO, ECDC, and CDC requirements, which is linked to the European surveillance portal for infectious diseases and the WHO FluNet global network with weekly reporting year-round. This system also includes the National Influenza Centre of the Republic of Moldova, which was recognized and approved in 2013 by WHO and is certified as a member of the Global Influenza Surveillance and Response System (GISRS).

CONCLUSIONS

1. During the 2014/2015 to 2022/2023 epidemic seasons, ILI exhibited low to medium intensity in the epidemiological process, while ARI displayed all levels of intensity from low to very high. Influenza-like illness, acute respiratory infections, and severe acute respiratory infections predominantly affected children in the 0-14 age group.

2. Over the last nine epidemic seasons, a large number of nasopharyngeal exudate samples have been investigated for influenza viruses, of which 12.7% were positive for influenza viruses A(H1N1) pdm09, A(H3N2), and B. There were no positive results for influenza in the 2020/2021 season.

3. According to the antigenic structure, the isolated influenza viruses are similar to the influenza virus strains that fit the phylogenetic tree constructed during the nominated period. The isolated influenza virus strains were susceptible to the antiviral drugs oseltamivir and zanamivir.

4. The results demonstrate the need to strengthen the system of epidemiological surveillance and response to ILI, acute respiratory infections, and severe acute respiratory infections existing in the republic in order to mitigate the socio-economic impact on the health system and the national economy.

5. Continuous epidemiological and virological surveillance provides crucial information for interventions depending on epidemiological evolution at the national level and allows prompt and targeted interventions if needed. In this way, health authorities can implement public health measures adapted to the situation, helping to limit the spread of infections and protect public health.

CONFLICT OF INTEREST

The author does not declare any conflict of interest.

ETHICAL APPROVAL

The article was not approved by the Ethics Committee because it does not contain ethical risks.

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EVALUATING THE EFFICACY OF SIMULATION-BASED TRAINING ON CLINICAL COMPETENCY TRANSFER

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Introduction. Efficient learning transfer is crucial for training specialists to ensure the provision of quality medical services. This study investigates the impact of simulation-based training on the transfer of medical students' clinical competencies in parenteral drug administration: subcutaneous (SC), intramuscular (IM), and intravenous (IV).

Material and methods. Using a quasi-experimental design, the research incorporates pre- and post-intervention testing, observational assessments, and a satisfaction survey to evaluate the educational impact.

Results. The results indicate significant improvements in theoretical knowledge and practical skills post-intervention, with notable score increases for all types of injections (SC: from 50% to 83.33%, IM: from 57.14% to 85.71%, IV: from 42.86% to 85.71%; $p<0.001$). Similarly, improved clinical performance was demonstrated, highlighting the transferability of the learned competencies to real settings. Student satisfaction recorded a score of 4.71 (95% CI 4.64; 4.79), reflecting strong acceptance and appreciation of the training.

Conclusions. The study emphasizes the crucial role of simulation in competency-based medical education, bridging the gap between theoretical knowledge and practical application, and advocates for the systematic integration of simulation into the medical curriculum, suggesting future research directions for optimizing the design and implementation of simulations.

EVALUAREA EFICACITĂŢII FORMĂRII BAZATE PE SIMULARE ASUPRA TRANSFERULUI DE COMPETENŢE CLINICE

Introducere. Transferul eficient al învăţării este esenţial pentru formarea specialiştilor, în vederea asigurării serviciilor medicale de calitate. Acest studiu investighează impactul instruirii bazate pe simulare asupra transferului competenţelor clinice ale studenţilor la medicină, în administrare parenterală a medicamentelor: subcutanată (ASC), intramusculară (AIM) şi intravenoasă (AIV).

Material şi metode. Folosind un design cvasi-experimental, cercetarea integrează testări pre şi post-intervenţie, evaluări observaţionale şi un sondaj de satisfacţie, pentru a evalua impactul educaţional.

Rezultate. Rezultatele indică îmbunătăţiri semnificative în cunoştinţele teoretice şi abilităţile practice post-intervenţie, cu creşterea scorurilor, în mod notabil, pentru toate tipurile de injecţii (ASC: de la 50% la 83,33%, AIM: de la 57,14% la 85,71%, AIV: de la 42,86% la 85,71%; $p<0,001$). De asemenea, s-a demonstrat o performanţă clinică îmbunătăţită, evidenţiind transferabilitatea competenţelor achiziţionate în condiţii reale. Satisfacţia studenţilor a înregistrat scorul de 4.71 (IÎ 95% 4,64; 4,79), reflectând o acceptare şi apreciere puternică a instruirii.

Concluzii. Studiul subliniază rolul primodial al simulării în educaţia medicală bazată pe competenţe, asigurând conexiunea între cunoştinţele teoretice şi aplicarea practică, şi susţine integrarea sistematică a simulării în curriculum medical, sugerând direcţii viitoare de cercetare, pentru optimizarea designului şi implementării simulărilor.

INTRODUCTION

Training future specialists with essential clinical competencies is fundamental, representing the core of Competency-Based Medical Education (CBME) (1). Increasingly, scientific evidence suggests that new teaching strategies have a positive impact on the efficiency of educational events (2). Simulation-based training has emerged as a vital component of CBME, bridging the gap between theoretical knowledge and the practical application of skills in a controlled, risk-free environment. However, it is still necessary to comprehensively evaluate how these simulated experiences translate into clinical competencies in the real work environment. This gap in research underscores the need not only to highlight the effectiveness of simulation-based training but also to outline the mechanisms through which these competencies are transferred and retained over time, contributing to the broader discourse on the evolution of pedagogies in medical education.

An increasing number of studies have shown that simulation-based training enhances learning outcomes, develops clinical skills, and boosts student confidence, ultimately leading to better patient care (3). Thus, Nuzzo and colleagues have demonstrated that medical students trained through simulation-based methods show significantly superior clinical competencies, evidenced by training outcomes including at objective structured clinical examinations (OSCE), compared to their peers who did not experience simulation (4). Comparative studies have shown that simulation-based learning is more effective than traditional lecture-based approaches, leading to greater knowledge retention and higher student motivation (5, 6, 7). Zendejas and colleagues have analyzed specialty studies regarding the influence of simulated training on patients cared for or assessed by physicians trained through this method and determined that there is evidence of a beneficial impact (8). This efficacy can be attributed to various features of the educational intervention design, such as deliberate practice, feedback, debriefing, high fidelity of the simulation, similarity of environments, etc., which are crucial for successful learning transfer (9, 10). Medical simulation helps learners understand that they can achieve good results when performing various procedures correctly. The confidence given is built on four main elements: personal experiences of success, observing the success of others,

receiving encouragement from evaluators, and one's own physical reactions (11).

Trainees can acquire and retain clinical competencies using simulators but sometimes demonstrate a limited ability to transfer them to situations other than training settings (12). Despite the proven benefits of simulation-based training, there remains a gap in understanding how such training affects the transfer of clinical decision-making skills and the professional preparation of nurses, indicating a need for further research in this area (13). However, some interprofessional simulation experiences have shown prospects not only in improving short-term perceptions of competencies but also in facilitating the transfer of distance learning experiences to clinical environments, thus underlining the value of simulation in promoting collaborative practice among medical professionals (9, 14). This effect might be due to the fact that the transfer of knowledge and competencies to the practical environment is a complex process that depends on at least three categories of factors – the personal characteristics of the trainee, the design of the educational intervention, and factors related to the work environment (15). In other words, developing professional competencies through targeted specific interventions represents just one component of this complicated ensemble, but it is one of the most crucial.

The role of medical simulation extends beyond mere skill acquisition, providing learners with authentic scenarios that mirror real-life situations (16). This experiential learning environment not only increases the confidence of the learners but also minimizes the risk of medical errors in subsequent professional activities, contributing to safer patient care (17, 18). Various simulation techniques, such as the use of standardized patients and manikins, are crucial for the development of clinical competence and adherence to professional standards, fundamental in achieving accurate diagnosis and effective treatment (19, 20, 21). Simulation-based training has a significant impact on the development of technical skills (22, 23, 24), and it also aids in the formation of non-technical skills (23, 25, 26), which enhances the clinical performances of trained medical specialists. The incorporation of simulation into medical training has been associated with sustained

improvements in clinical competencies, highlighting the comprehensive nature of simulation training in addressing sets of competencies (17, 19, 22, 27, 28). Such findings advocate for the integration of simulation-based methodologies into medical curricular programs at all levels of professional medical training, to design more effective educational interventions.

Systematic evaluation of the impact of training programs is essential for determining their effectiveness, covering a wide spectrum from immediate reactions to long-term effects, according to the principles established by Kirkpatrick (29). For analysing clinical competencies and participant satisfaction, various methods have proven effective, including statistical analysis (30), online surveys (31), and the use of checklists (32). Additionally, assessing participant satisfaction and the impact of training on individual beliefs in performing clinical procedures aligns with Bandura's theory of self-efficacy, which suggests that confidence in one's abilities to succeed in specific situations can enhance performance (11).

Overall, simulation has been validated as a means to enhance the performance of medical students in clinical settings, with evidence indicating its efficacy, including in improving clinical competencies at OSCE stations (4, 33, 34). However, the effective integration of simulations into the medical education curriculum remains a challenge, given the need for significant resources, specialized equipment, and faculty training. Additionally, future research should explore ways to optimize the design and implementation of simulations to maximize learning transfer, including the use of emerging technologies such as virtual and augmented reality (35, 36, 37).

The aim of this study was to comprehensively evaluate the impact of a simulation-based educational intervention on the acquisition of transferable clinical competencies, using the example of parenteral medication administration skills (subcutaneous, intramuscular, and intravenous injections), which is in line with current methodologies in professional training. The specific objectives can be summarized as follows: (i) Evaluate the efficacy of a structured simulation-based educational intervention in improving medical students' knowledge retention in performing injections; (ii) Analyze the impact of the educational intervention on practical skills; (iii) Determine the transferability of skills acquired through

simulation into clinical practice; (iv) Assess participant satisfaction with the training program and its impact on self-confidence in performing clinical procedures, which may suggest that confidence in the ability to succeed enhances performance in specific situations.

The article represents an extension and completion of the preliminary results of the study presented at the conference "Medical simulation – a look into the future" in 2021, published in the abstract collection of the same conference (38). While the previous publication provided an initial perspective on the research outcomes, this study deepens the analysis and includes a complete set of data, offering a more comprehensive picture and significant additional details that were not previously reported. Therefore, this article not only enhances the understanding of the effectiveness of simulation-based training but also highlights new findings that underscore the importance of our research in the field of knowledge and skill transfer, validating and expanding preliminary observations for further research and practical applications.

MATERIAL AND METHODS

The conducted study has a quasi-experimental design and comprised a structured educational method in four stages for learning a competency, using medical simulation. Participants involved were students (n=43) in their 3rd and 4th years of the Medicine program at Nicolae Testemiţanu State University of Medicine and Pharmacy. None of them indicated having post-secondary medical education (college) and each enrolled in the participation list due to the lack of specialized and comprehensive training on parenteral medication administration techniques. Recruitment was based on a public call for participation, and inclusion criteria were: being a Medicine student and agreeing to voluntarily participate in the study. Conducted from February 10 to 14, 2020, the study took place at the University Center for Simulation in Medical Training for basic training, and at the University Clinical Center for Primary Medical Care for practicing maneuvers in a clinical environment. Participants were informed about the objectives and methodology of the study and ultimately, all voluntarily consented to be involved.

The training focused on the parenteral administration of medicinal preparations through three basic techniques – subcutaneous, intramuscular,

and intravenous, and was structured into two main components, a theoretical one followed by a practical one. The theoretical part of the training involved the distribution of educational materials and the theoretical study of the procedures to be performed, taking place in a single session in the form of a seminar. The practical part, in turn, consisted of two phases. The first phase included the use of task-training simulators combined with role-playing methods to replicate real conditions, where participants were trained through a four-step didactic process – demonstration, deconstruction, comprehension, and performance (39). The second phase took place in a controlled and safe clinical environment on a live person, a few hours later. As patients, the students themselves were involved, who had given their consent for subcutaneous, intramuscular, and intravenous injection procedures to be performed. It should be noted that at all stages of training, participants received feedback from the instructors.

Data collection was conducted through three measurement methods: A. Theoretical evaluations – an initial "pre-test" (TE no.1) and a final "post-test" (TE no.2), with the same set of questions; B. Observational evaluations – conducted at the end of the training, in the educational environment (OE no.1) and during practice in a real clinical setting (OE no.2), using the same checklist; C. Participant satisfaction evaluation (SE) was conducted with an anonymous Likert-type questionnaire with a 5-point scale, from "Strongly Disagree" (1 point) to "Strongly Agree" (5 points) (fig. 1). All research instruments (theoretical test, observational evaluation checklist, and questionnaire) were developed specifically for this intervention within the study. Data processing and analysis were performed using CAE Learning-Space®, Epi-InfoTM7, Microsoft Office Excel 2013, R Studio, and SPSS 26.
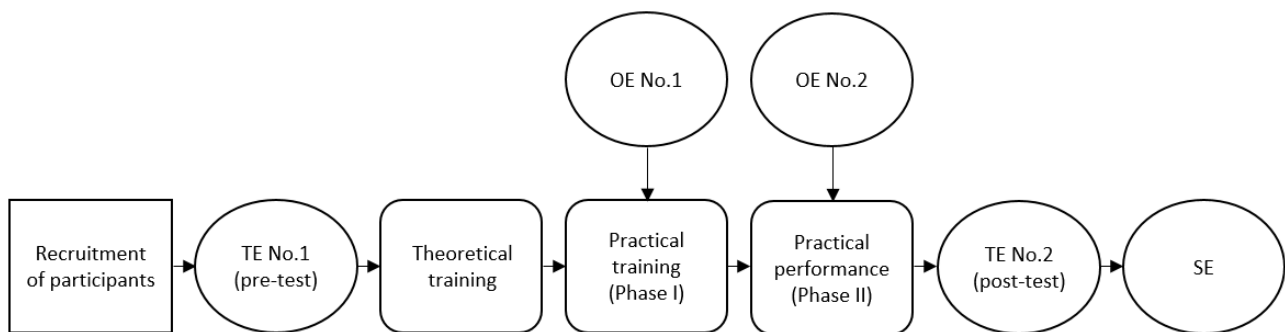
Figure 1. The design and stages of the study.

To demonstrate the positive effect of the educational intervention on the level of knowledge and skills, a significant difference is expected between the pre-/post-test evaluation scores (where TE no.1 < TE no.2) and satisfactory results ("good" and "very good" according to national and European grading systems) in the observational evaluation at the end of the training (OE no.1). Additionally, to establish the positive transfer of competencies acquired in the learning environment to the clinical work environment, the score recorded at the end of the training should be lower than that recorded in the clinical setting. Also, to assess the reaction and any suggestions regarding the intervention, participants were surveyed anonymously.

RESULTS

A. The results of the comparative theoretical

evaluations pre-/post-test showed a statistically significant improvement in knowledge of injection administration for all three types studied. Specifically, competencies for administering subcutaneous injections recorded an average increase from 50% to 83.33% after the intervention, with a Wilcoxon signed-rank test indicating a significant difference (Vw=12.00; p<0.001) and an impressive effect size, estimated by the rank biserial correlation test (r=-0.97). The narrow confidence interval (CI 95% 0.99; 0.95) confirms the strong positive impact of the intervention (fig. 2).

Similarly, the IM administration skill demonstrated an average improvement from 57.14% to 85.71%, with statistical results highlighting a significant improvement (Vw= 0.00; p<0.001) (fig. 3).

A similar situation is observed in the case of IV ad

ministration. An increase in scores from 42.86% to 85.71% was noted, with an extremely significant improvement (Vw=0.00; p<0.001), highlighting a direct and strong impact of the training on the practitioners' knowledge (fig. 4).
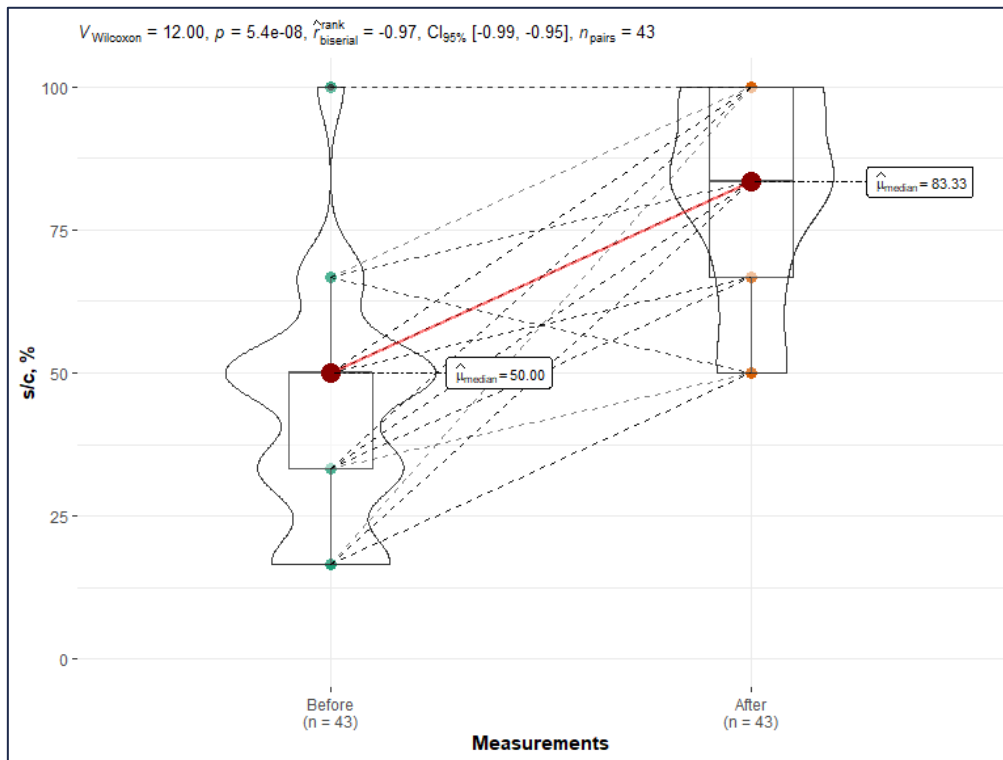
Figure 2. The comparative results of the theoretical evaluation (TE no.1 vs. TE no.2), for the SC administration skill.
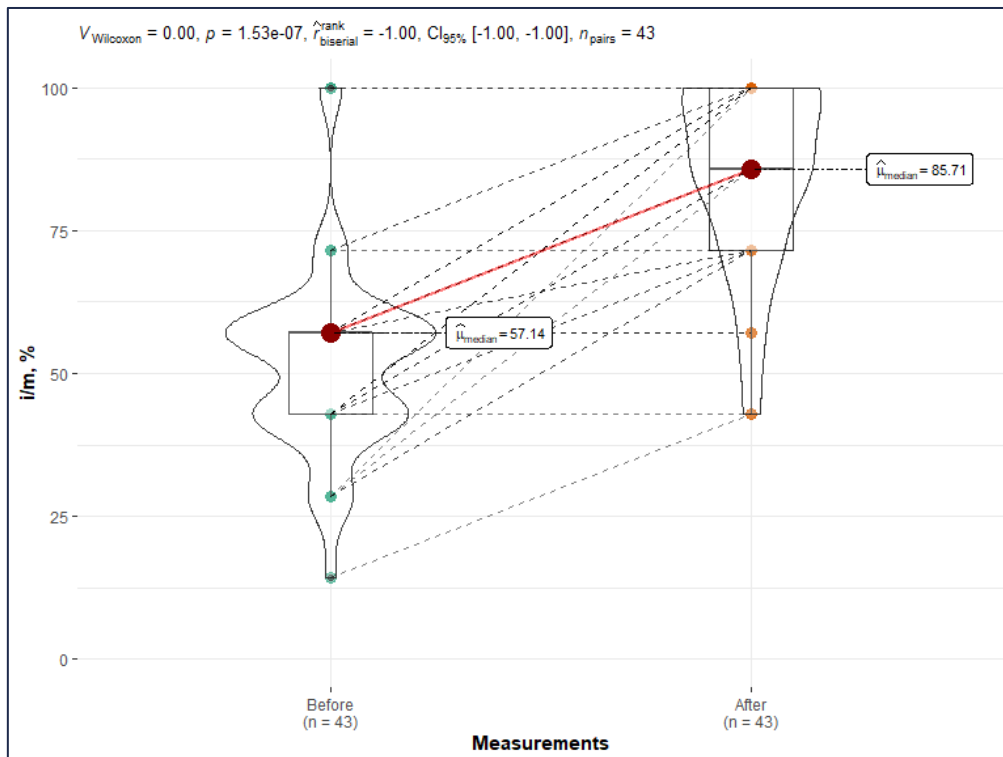
Figure 3. The comparative results of the theoretical evaluation (TE no.1 vs. TE no.2), for the IM administration skill.
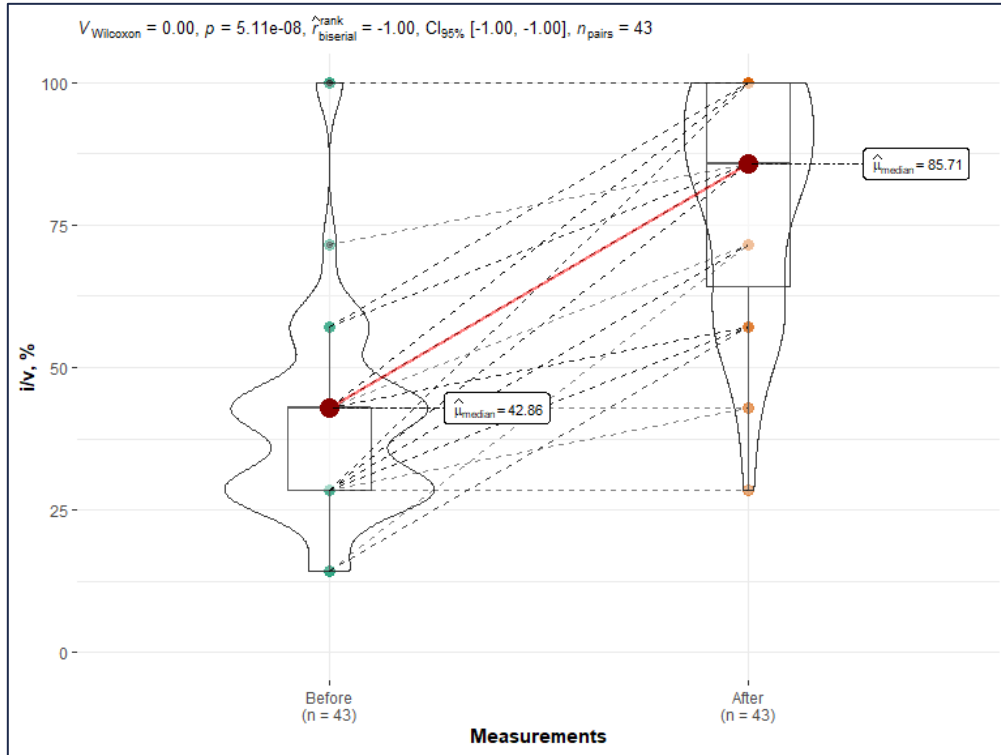
Figure 4. The comparative results of the theoretical evaluation (TE no.1 vs. TE no.2), for the IV administration skill.

Figure 5. The comparative results of the observational evaluations (OE no.1 vs. OE no.2), for the IM administration skill.

B. The analysis of observational evaluations based on the checklist, immediately after the training phase (OE no.1), showed good performance results for all three learned skills.

Thus, the overall average score was: 81.09% (CI 95% 80.95; 81.22) for the SC administration maneuver, which corresponds to a grade of 8.5 or the range of 8.01-8.5, according to the national grading system (NGS) and a grade of B or "very good" as per the scale with grades recommended in the European Credit Transfer System (ECTS); 73.92%

(CI 95% 73.8; 74.04), or 7.5 according to NGS or C/"good" according to ECTS, for the skill of performing intramuscular injections; and for the IV administration skill, similar to the first maneuver – 80.57% (CI 95% 80.46; 80.69), or a grade of 8.5 according to NGS and B/"very good" according to the ECTS system.
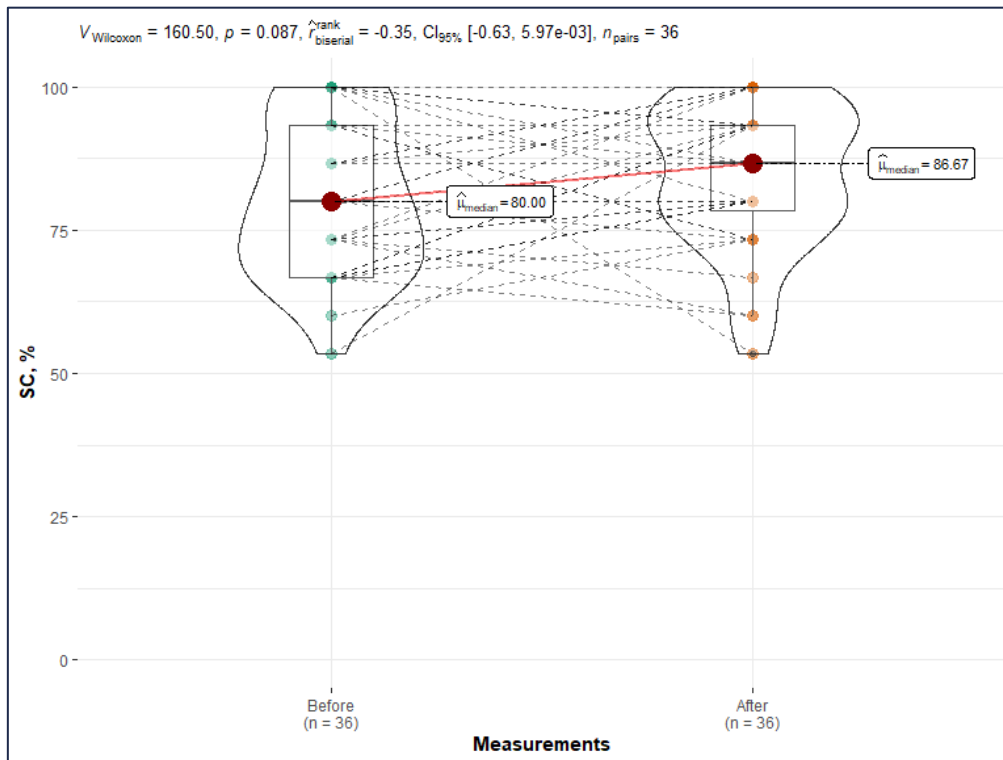
Figure 6. The comparative results of the observational evaluations (OE no.1 vs. OE no.2), for the SC administration skill.

The comparative analysis of the results of observational evaluations, immediately after the training phase (OE no.1) and during practice in a clinical setting (OE no.2), conducted based on the median scores, highlighted a significant improvement in the competencies for performing intramuscular injections from 71.43% post-training to 85.71% in the clinical setting (p=0.004). The effect size was estimated by the rank biserial correlation test (r=-0.66) (fig. 5).

For the other two skills, the comparative results proved to be more modest. For SC administration, an increase in median performance was observed from 80.00% in the educational setting to 86.67% in the clinical setting (p=0.087) with a medium effect size (r=-0.35) (fig. 6), and for IV administration, it increased from 82.35% post-training to 88.24% during clinical practice (p=0.1), with a medium effect size of the biserial correlation

(r=- 0.33) (fig. 7).

The detailed comparative analysis (OE no.1 vs. OE no.2) for the SC administration skill revealed that for most criteria (C#) on the evaluation checklist, there were either improvements or no significant changes in the percentage outcomes. The most notable improvements were observed for items C8 (control aspiration), C11 (use of sharps disposal container), and C14 (cleaning the work area after the procedure), suggesting that the training had a positive effect on these specific criteria. It is worth noting that there was also a notable decrease in C9 (slow injection of the pharmaceutical) during the procedure in a clinical setting. The comparison of observational evaluation results for IM administration showed higher positive response rates in OE No.2 compared to OE No.1 for most items. The largest positive differences were recorded for C9 (rapid withdrawal of the needle

with application of a sterile pad at the injection site), C10 (use of sharps disposal container), C11 (securing the applied pad with adhesive tape), C13 (cleaning the work area after the procedure), C14 (hand hygiene at the end of the procedure). For items C2 (communication with the patient and explaining the procedure) and C5 (asking the patient to relax the muscles in the injection region), a decrease in the rate of positive ratings was noted. In the case of the IV administration maneuver, the data shows improvements in cer-

tain criteria following the educational intervention, such as C7 (inserting the needle at a 35-degree angle), C8 (inserting the needle into the vein 3-5 mm with the syringe and needle position secured), with the most significant improvements observed for C6 (stabilizing the vein by longitudinal traction of the skin) and C10 (loosening of the venous tourniquet). However, some areas showed a decrease in scores after the intervention in a real clinical setting, such as C17 (hand hygiene after the procedure).

$V_{Wilcoxon} = 176.00$, $p = 0.100$, $\hat{r}^{rank}_{biserial} = -0.33$, $CI_{95\%}$ [-0.62, 0.03], $n_{pairs} = 36$
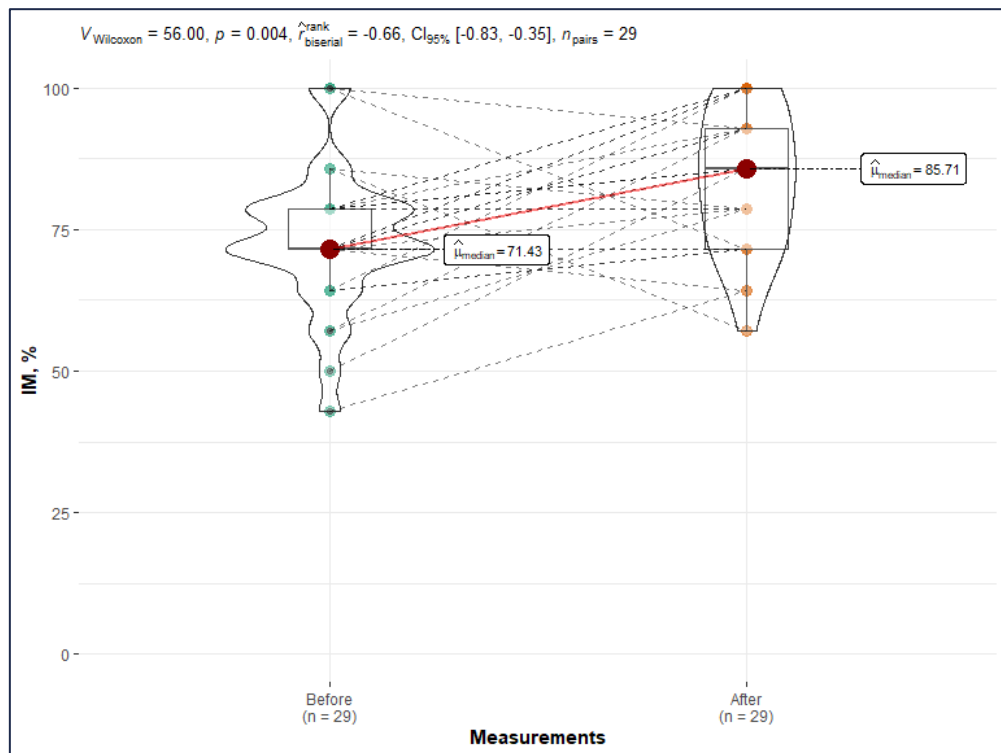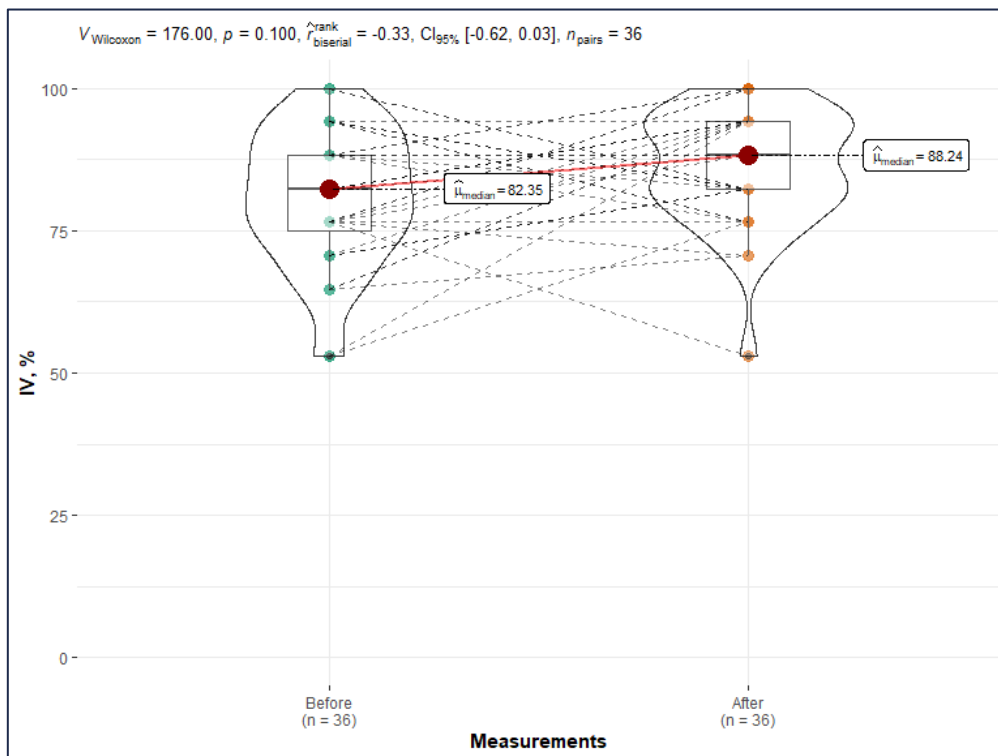
Figure 7. The comparative results of the observational evaluations (OE no.1 vs. OE no.2), for the IV administration skill.

The analysis of participant satisfaction in the study, conducted using a Likert-type questionnaire, reflected a high level of overall satisfaction. The simulation-based training method was particularly appreciated, with an average score close to the maximum possible of 4.71 points (CI 95% 4.64; 4.79), indicating strong acceptance and appreciation of this educational intervention. The total average score obtained by participants was 56.53 points out of a maximum of 60.00 points (CI 95% 55.65; 57.42), highlighting a high degree of satisfaction, which validates the reliability and consistency of the results obtained. The per-question analysis of the satisfaction questionnaire demonstrated that all evaluation criteria rec-

orded significant scores, ranging from 4.33 (minimum score) to 4.98 (maximum score) out of a possible 5.00, which corresponds to the rating of "Strongly Agree" (4.30–5.00). Notably, the items with the highest scores stood out, especially: "Q5. Medical training through simulation deserves to be promoted and recommended" with a score of 4.98 (CI 95% 4.93; 5.02), followed by "Q6. I would opt for other courses using the method of medical training through simulation in the future" – 4.95 (CI 95% 4.89; 5.02) and "Q2. The method of training through simulation motivates me to develop professionally" – 4.93 (CI 95% 4.85; 5.01). The lowest scores were recorded for "Q10. The number of repetitions of practical maneuvers during

training was sufficient to master the respective competency" – 4.33 (CI 95% 4.10; 4.55) and "Q12. I believe that after completing this course I will possess the necessary practical skills to correctly perform the medical interventions taught", scoring 4.33 (CI 95% 4.08; 4.57).

DISCUSSIONS

The specialized literature emphasizes the crucial role of simulation methods in ensuring the transfer of learning in medical education. Simulations not only improve clinical competencies and students' self-confidence but also facilitate the application of theoretical knowledge in practical contexts. The study is quasi-experimental in order to investigate the effectiveness of simulation-based training regarding the transfer of clinical skills among medical students. However, there are some potential limitations related to the study design, such as the lack of a control group or potential biases in observational evaluations.

The data obtained clearly show a substantial improvement in the theoretical knowledge of medical students regarding injection techniques after the educational intervention. The significant statistical value and large effect size underscore the effectiveness of the "four-step" teaching approach based on simulation methods. The increase in the median score at the theoretical evaluations by over 30 percentage points, together with the very narrow confidence interval for the effect size, provide solid evidence that the intervention was highly effective. These results suggest that such educational interventions, which combine theoretical and practical training with simulation, are highly beneficial in improving the theoretical knowledge of medical students in this field. Additionally, the post-training observational evaluations indicated good performances in administering subcutaneous, intramuscular, and intravenous injections, with average scores reflecting solid competencies. The scores ranged from "good" to "very good" according to national and European grading systems, highlighting the effectiveness of the training in developing the targeted practical skills.

A key aspect addressed in this article is the impact of the educational intervention on the application of practical skills in a real clinical context. Repeated observational evaluations in the clinical setting (OE no.2) aimed to determine whether and how the skills learned in the training environment are transferred and applied in actual prac-tice. Anticipating the outcomes of the measurements, we could hypothetically assume a range of possibilities. They might be modest, remain unchanged or insignificantly changed, or could be improved compared to the results obtained at the end of the training (OE no.1). In the first scenario, when the clinical environment observations indicate inferior performances, this suggests that the competencies developed in the training environment are too narrow and specific, inadequate for more varied contexts and not transferable, or that external factors, such as working conditions (including stress, material resources, work environment conditions), may hinder the effective transfer of skills (40). However, this assumption was contradicted by the findings of our study. At the opposite end of the spectrum is the case where clinical performances exceed the results achieved at the conclusion of the initial training, as in the case of the intramuscular administration competency in our study, where the increase in the median score at OE no.2 exceeded by approximately 14 percentage points (p=0.004), with an effect size of -0.66. In this context, it can be deduced that certain competencies significantly benefit from the specific educational intervention through simulation, not only reaching the desired performance level but also enhancing the skill transfer effect. This effect can also be attributed to repeated practice, a principle supported by research in the field (41). There are cases where clinical performance is similar or only slightly different from that in the training environment, as seen for subcutaneous and intravenous administration competencies. At first analysis, it might be misinterpreted that the training had no effect. However, this finding actually indicates that the acquired skills are transferable and applicable in real contexts. That is, the good results verified at the evaluations at the end of the training are maintained in the evaluations conducted in the real clinical environment, demonstrating the effectiveness and positive outcomes of the educational intervention. This suggests that the learned competencies are well generalized and can be effectively implemented in clinical practice (42). Nevertheless, a detailed analysis of the results of observational evaluations might suggest increasing attention to certain aspects of practical training, such as effective communication with the patient and proper management of post-injection procedures, through an adjusted or additional pedagogical approach to maximize the efficiency of training and ensure positive transfer.

The analysis of participant satisfaction in the study reflected a high level of overall satisfaction (score = 4.71 points), with an average close to the maximum possible score (5.00 points), indicating strong acceptance and appreciation of this component of the course. The average total score of 56.53 (CI 95% 55.65; 57.42) out of a maximum of 60 points underscores a high degree of satisfaction, and the narrow confidence interval validates the reliability and consistency of the data obtained. These results reinforce Bandura's (1977) theory on self-efficacy (11), demonstrating that confidence in one's own abilities can be significantly improved through deliberate practice in a controlled, risk-free environment, which facilitates learning and the transfer of skills in real clinical contexts (6, 8).

In line with previous studies that have highlighted the ability of simulation to improve clinical competencies and facilitate the transfer of learning, this study adds concrete evidence regarding the effectiveness of simulation in training for injection administration. Considering the significant improvements in performance and the substantial impact indicated by the effect size, it is clear that this educational strategy deserves to be implemented widely in professional training programs. Additionally, our study demonstrates enhanced effectiveness of simulation training methods when combined with role-playing and the "four-step" teaching approach to a skill, highlighting the applicability and importance of this strategy in the context of contemporary medical education. There is a clear need for deliberate integration of simulations into medical educational programs, not only to enhance technical skills but also to strengthen confidence and non-technical competencies, vital for ensuring high-quality and safe clinical practice (27, 28).

Although the widespread implementation of simulation-based training in the medical curriculum can face some difficulties, such as the need for substantial resources, specialized equipment, and appropriate teacher training, the findings of the study underscore its importance in cultivating clinical competencies in future health professionals. Further research should aim to identify the most effective strategies to optimize the implementation of simulations, to facilitate the transfer of knowledge and clinical competencies into the real-world environment.

CONCLUSIONS

1. The study results showed statistically significant improvements in theoretical knowledge, demonstrating the strong positive impact of the educational intervention.

2. Post-training observational evaluations indicated good performances in administering subcutaneous, intramuscular, and intravenous injections, with average scores reflecting solid competencies.

3. The analysis highlighted that the competencies acquired through simulation were transferable and applicable in the real clinical environment.

4. Student satisfaction with the educational intervention was high, reflecting strong acceptance and appreciation of simulation-based training, with a positive impact on self-confidence and motivation for professional development.

CONFLICT OF INTEREST

The authors of the article deny the existence of any conflict of interest in the publication of this material.

ETHICAL APPROVAL

The research has been approved by the Research Ethics Committee, no. 59, April 2, 2018, at *Nicolae Testemitanu* State University of Medicine and Pharmacy, Republic of Moldova. All participants were informed about the objectives and methodology of the study and voluntarily consented to participate by signing.

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PARENTAL EDUCATION LEVEL ON THE DEVELOPMENT OF CHILDREN'S ORAL HYGIENE SKILLS

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Introduction. *Dental caries is a significant public health issue due to its high incidence among the population. While modern scientific research highlights effective treatments for this condition, prevention remains crucial, directly impacting health and quality of life, especially when early preventive habits are instilled. This study aims to explore how parents' educational levels and other factors influence the acquisition, use, and proper application of oral hygiene knowledge among primary school children.*

Material and methods. *The study surveyed 678 parents of primary school children across 9 administrative regions, including both urban and rural areas of the Republic of Moldova. It employed a randomized approach, involving respondents from all social groups.*

Results. *This study found that parental education level is a crucial factor influencing the proper development of oral hygiene skills, which can prevent dental conditions. Higher parental education was positively associated with urban living environment and family income. Additionally, well-educated parents tend to visit dentists more regularly, which also reflects in better oral hygiene practices at home for their children.*

Conclusions. *Children's oral hygiene habits are strongly shaped by socioeconomic factors and the care parents show. Increasing parental education levels, whether in urban or rural settings, plays a pivotal role in fostering correct dental hygiene practices early in life, thereby ensuring long-term oral health habits into adulthood.*

Cuvinte-cheie: *periaj dentar, igiena orală, părinți, educație.*

NIVELUL DE EDUCAȚIE AL PĂRINȚILOR PRIVIND DEZVOLTAREA ABILITĂȚILOR DE IGIENĂ ORALĂ LA COPII

Introducere. *Caria dentară este o problemă importantă de sănătate publică din cauza incidenței înalte în rândul populației. În pofida faptului că studiile științifice moderne demonstrează existența tratamentului eficient a acestei maladii, totuși profilaxia rămâne a fi primordială, având un impact direct asupra sănătății și calității vieții, în special dacă abilitățile de prevenire se cultivă încă din copilărie. Scopul studiului constă în determinarea rolului nivelului educațional al părinților și a altor factori care pot influența dobândirea, utilizarea și aplicarea corectă a cunoștințelor în igiena orală a copiilor din învățământul primar.*

Material și metode. *În cadrul studiului, au fost chestionați 678 de părinți ai copiilor din învățământul primar din 9 regiuni administrative – urbane și rurale ale Republicii Moldova. Studiul a fost unul randomizat, care a inclus respondenți din toate grupurile sociale.*

Rezultate. *În acest studiu am stabilit, că nivelul de educație al părinților este un factor esențial care determină dezvoltarea corectă a abilităților de igienă orală, capabile să prevină afecțiunile dentare. Creșterea nivelului de educație al părinților a fost asociată pozitiv cu mediul de trai urban și cu venitul familiei. Părinții cu un nivel ridicat de educație vizitează mai frecvent medicul stomatolog. Nivelul de educație a influențat pozitiv și parametrii legați de igiena orală a copilului la domiciliu.*

Concluzii. *Abilitățile de igienă orală ale copiilor sunt fortificate de factorii socio-economici și de atitudinea, grija pe care părinții le manifestă față de copii. Creșterea nivelului de educație al părinților din orice zonă geografică – urbană sau rurală influențează pozitiv formarea deprinderilor de igienă dentară corectă la copii, fiind astfel garantul sănătății orale la vârsta adultă.*

INTRODUCTION

Pre-pubertal children (6-11 years old) have an increased incidence of dental conditions, including dental caries. At the same time, this period is critical because it lays the foundation for lifelong oral hygiene skills and habits. During this period, children also begin school, where their ability to acquire knowledge can facilitate education and promote proper oral hygiene practices. However, the specialized literature data show that school-based education through traditional readings primarily increases knowledge about oral health, without significantly improving actual oral hygiene habits (1). A series of other factors, such as the socio-economic status of the parents, the family income, the gender of the parent, and the number of parents, can influence the child's oral hygiene skills (2). Currently, various programs aimed at preventing or treating oral diseases in children are being implemented across different countries, though their effectiveness and outcomes often remain controversial.

The purpose of this study was aimed to determine how parental education levels and other factors influence children's acquisition, use, and proper application of oral hygiene knowledge in primary school. This study is particularly crucial given Moldova's significant socio-economic decline, which adversely affects the education levels of its population. The findings can help set priorities for medical care planning and the implementation of preventive measures.

MATERIAL AND METHODS

Research design. To ensure data quality, a total of 678 printed questionnaires were randomly distributed across nine different urban and rural administrative regions in the Republic of Moldova. The questionnaires were distributed in primary and secondary schools, targeting parents of primary school children. Additionally, 37 questionnaires were distributed to parents accompanying their children to the Municipal Dental Center for Children in Chisinau during dental visits. The questionnaire contained a series of multiple-choice questions designed to elicit information relevant to the study. 6 questionnaires were excluded from the analysis due to their incorrect completion. Finally, the analyzed cases (672) included the opinions of 310 respondents from rural and 362 from urban regions of Moldova.

The study was carried out between December 2022 and March 2023.

In the "General Information" chapter, parents provided details on various family characteristics, including:
- Gender of the parent and child (1 for female, 2 for male).
- Parents' education level: both higher education; one higher education and one professional background; one upper and one middle school education; both professional backgrounds; one professional background and one other; both middle school education; one middle school education and one incomplete middle school education; both incomplete middle school education (rated from 8 to 1)
- Living environment: rural or urban (1 for rural, 2 for urban).
- Family income: very low, low, medium, high, very high (rated from 1 to 5).
- Child's age: 6-11 years.
- Whether they have ever taken the child to the dentist: yes (1) or no (0).

In the "Guidelines for Your Child's Oral Hygiene at Home" chapter, parents were asked to provide answers to the following questions:
- How often does your child brush the teeth each day? Response options included: I don't know; never; once in the morning; once in the evening; twice; three times or more (from 0 to 4).
- What type of toothbrush does your child use? Options included: manual; electric; sonic (from 1 to 3), with multiple selections allowed.
- How frequently do you replace your child's toothbrush? Options ranged from: when it's worn out; monthly; every 3 months; every 6 months (from 1 to 4).
- What brushing techniques does your child use? Options included: I don't know; horizontal; vertical; circular; mixed (from 1 to 4).
- How long does your child typically brush the teeth? Response choices included: I do not know; up to one minute; up to two minutes; more than two minutes (from 1 to 4).
- Have you ever taught your child how to floss and supervised them doing it? Responses included: never; no, because I didn't see the need; yes, once a week; yes, once a day; another response (from 1 to 5).

- Does your child brush the tongue after brushing the teeth? Options included: I don't know; no; yes (from 1 to 3).
- Do you use mouthwash with your child? Answer options included: I don't know how to use it; we don't use it; yes, daily after brushing their teeth; yes, instead of brushing teeth (from 1 to 5).

When asked about their participation in oral hygiene information sessions conducted by the dentist, either at school or elsewhere, respondents were given the option to choose yes or no. Similarly, they were given the same response options when asked whether children should be informed about oral health at school. The final question related to parents' opinions on whether their child's oral hygiene skills had improved due to attending school (yes/no).

Each of the respondents completed only one questionnaire. The study was a randomized one, which included respondents from all social strata. Incomplete forms were excluded from the analysis.

Statistical analysis involved recording and organizing responses from questionnaires using MS Access 2007. WINSTAT software (R. Fitch Software, DE), being focused on determining the frequency and incidence of each response type. Additionally, Spearman's rank correlation coefficient (r_s), was used to examine statistical associations, while differences between groups were assessed using the Chi-squared test. Linear regression analysis with 95% confidence interval was applied, with correlation (R) and determination coefficients (R^2). Evans' (1996) recommendations were followed to interpret the degree of association: correlations were categorized as very weak (0-0.19), weak (0.20-0.39), moderate (0.40-0.59), strong (0.60-0.79), or very strong (0.80-1.0) (3).

A statistically significant threshold value of p≤0.05 was applied across all analyses. Bibliographic references were managed automatically using Zotero 6.0.18 software integrated with Word 2007 and Chrome (www.zotero.org).

RESULTS

This study revealed that children primarily visit the dentist with mothers (526 or 78.3%) compared to fathers (146 or 21.7%). Girls constituted 353 (52.5%), while boys made up 319 (47.5%) of the sample. Respondents' educational backgrounds were categorized as follows: higher education only – 130 (19.3%); higher education and professional backgrounds – 76 (11.3%); upper and middle school education levels – 86 (12.8%); professional backgrounds only – 100 (14.9%); professional backgrounds and middle school education – 84 (12.5%); middle school education only – 121 (18%); middle school and incomplete middle education – 40 (6%); incomplete middle school education only – 35 (5.2%). The majority indicated an average income (458 or 68.2%), followed by low-income (137 or 20.4%), very low-income (35 or 5.2%), high-income (26 or 3.9%), and very high-income (16 or 2.4%) families. Most of parents (573/85.3%) reported joint visits with their child to the dentist as part of their medical history.

Of the total number, 384 children (57.1%) reported brushing their teeth twice daily, both in the morning and evening. Following them are children who brush once in the morning (146 children, 21.7%), once in the evening (91 children, 13.5%), never (19 children, 2.8%), and three times or more per day (13 children, 1.9%). However, 19 parents (2.8%) admitted they do not know if their child brushes their teeth.

Manual toothbrushes were the most commonly used (506 children, 75.3%), followed by electric toothbrushes (116 children, 17.3%), sonic toothbrushes (2 children, 0.3%) and a combination of manual and electric toothbrushes (48 children, 7.1%).

About half of the respondents (319 children, 47.5%) reported changing the brush every 3 months, followed by those who change monthly (168 children, 25%), every 6 months (115 children, 17.1%), when the brush is damaged (69 children, 10.3%), or at random intervals (1 child, 0.1%).

While brushing their teeth, 314 parents (46.7%) observed that their child used mixed movements, 132 (19.6%) noticed rotary movements, 58 (8.6%) observed only horizontal movements, 57 (8.5%) noted vertical movements, 16 (2.4%) – combined horizontal and vertical movements, 8 (1.2%) – vertical and rotary movements, 1 (0.1%) – horizontal and rotary movements, and another one (0.1%) noticed rotary and mixed movements. Meanwhile, 85 parents (12.6%) admitted that they do not know how their child brushes their teeth.

Among all respondents, 271 (40.3%) timed their child's brushing at 2 minutes, 187 (27.8%) at 1 minute, 143 (21.3%) more than 2 minutes, and 71 (10.6%) were uncertain.

Half of the parents (342, 50.9%) stated that neither they nor their children use dental floss, followed by 119 (17.7%) who reported using it once a day, 106 (15.8%) who had used it but did not like it, 65 (9.7%) who used it once a week, and 39 (5.8%) who selected another option without specifying. Only one respondent (0.1%) reported using floss as needed.

The number of children who brush their tongues after brushing their teeth (296/44%) was close to those who do not clean their tongues (270/40.2%). About 15.8%, or 106 parents, indicated they do not know if the child brushes the tongue.

Another dental hygiene product, mouthwash, was not used by the vast majority of respondents (412/61.3%), followed by 198/29.5% who use it after brushing, 40/6% instead of brushing, 21/3.1 % who do not even know how to use it and one person (0.1%) mentioned sometimes using mouthwash, as needed.

More than half of the parents (427/63.5%) indicated that they did not participate in sessions related to oral hygiene, in contrast to 245/36.5% who answered positively. However, most respondents (640/95.2%) considered it necessary to inform schoolchildren about oral hygiene during their school years. Moreover, 519/77.2% of parents noticed that oral hygiene skills changed after they started attending school. Among them, 500/96.3% rated these changes as significant, scoring them above 5.

The correlation analysis highlighted, in all cases, statistically significant associations between the parents' level of education and the parameters included in the study.

Thus, higher levels of parental education were positively correlated with living in urban areas (r_s=0.41, p=0.000, R=0.41, R^2=0.17) and family income (r_s=0.35, p=0.000, R=0.37, R^2=0.13). Parents with higher education levels were also more likely to emphasize their children's dental visits (r_s=0.29, p=0.000, R=0.31, R^2=0.09). The level of education also positively influenced the parameters related to the child's oral hygiene at home, including frequency of brushing per day (r_s=0.24,

p=0.000, R=0.26, R^2=0.07), the type of toothbrush used (r_s=0.27, p=0.000, R=0.27, R^2=0.07), brush change frequency (r_s=0.13, p=0.0003, R=0.16, R^2=0.03). Also, the parents' educational level impacted oral hygiene behaviors such as brushing technique (r_s=0.18, p=0.000, R=0.16, R^2=0.03), duration of brushing (r_s=0.25, p=0.000, R=0.26, R^2=0.07), and tongue cleaning (r_s=0.21, p=0.000, R=0.21, R^2=0.04). Children of parents with higher education were more likely to use dental floss (r_s=0.30, p=0.000, R=0.27, R^2=0.07) and mouthwash (r_s=0.21, p=0.000, R=0.21, R^2=0.04) on a regular basis.

The studies positively influenced the parents' decision to participate in oral hygiene information sessions (r_s=0.21, p=0.000, R=0.21, R^2=0.05). Furthermore, the studies also positively impacted parents' decision to promote oral hygiene information courses within the school curriculum (r_s=0.06, p=0.05, R=0.06, R^2=0.00). At the same time, Parents with higher education critically examined the absence of these courses, noting that children's oral hygiene skills did not improve despite attending school (r_s=-0.16, p=0.000, R=0.16, R^2=0.02).

When comparing the values depending on the parent's gender, women were more likely than men to accompany children to dental visits and were also more attentive to certain aspects of oral care quality (tab. 1, tab. 2).

Moreover, respondents' place of residence emerged as a significant factor, revealing statistically significant differences in oral care quality (tab. 1, tab. 2).

Other factors, such as living environment, family income, and visiting the dentist with the child, also influenced oral hygiene skills, as demonstrated by varying degrees of statistical correlational associations (tab. 2).

DISCUSSIONS

Dental caries morbidity is one of the primary indicators in assessing the level of medical care and the population's health status. The World Health Organization recommends the implementation of prevention, detection, and treatment programs to avoid oral diseases (4). Untreated, these conditions can have systemic repercussions. Literature data indicate the influence of several factors on the caries incidence, including geographic locati-

on, dietary habits, water quality, and inadequate oral hygiene practices (5). Moreover, a 2011 study revealed that over half of children and adolescents in the Republic of Moldova seek dental care only during emergencies or when complications arise (6). This trend worsens with age, as approximately eight out of ten adults in Moldova suffer from tooth decay.

Table 1. Statistical differences between the analyzed parameters based on parental gender and place of residence via the Chi-squared test.

Variables	Parent's gender (χ^2)	Place of residence (χ^2)
Education level	**356.59**	**607.73**
Living environment	44.55	-
Family income	79.89	131.79
Visit with children	**321.01**	68.02
Frequency of brushing	65.55	72,01
Brush type	75.54	142.64
Brush change frequency	**219.06**	**400.56**
Type of brushing	**772.79**	**1054.20**
Brushing time	128.47	209.51
Flossing	**312.16**	**708.15**
Tongue brushing	103.67	163.78
Mouthwash use	**253.75**	**401.89**
Oral hygiene meetings	60.09	85.53
Need for information	5.74	14.58
School -acquired skills	36	79.34

Notes: Statistically significant differences (p≤0.05) were highlighted with **bold.**

This is the first study on the territory of the Republic of Moldova that considers socioeconomic factors that influence oral hygiene in children.

According to Gurav et al. (2022), school-based oral health education programs based on modern technologies and traditional courses could strategically promote oral health behavior in developed and developing countries (5). Nevertheless, the question remains whether these initiatives alone ensure satisfactory oral hygiene outcomes in children.

Poulton et al. (2002) present data indicating that the severity of tooth decay and periodontal diseases in childhood correlates with socioeconomic status, leading to long-term consequences (7). On the other hand, Castilho et al. (2013) consider that parents play a fundamental role in establishing oral hygiene practices for children, whether through direct teaching or by influencing their attitudes towards dental care (8).

A recent study conducted by Dumitrescu et al. (2022) in Romania showed that children whose parents have higher education levels are less likely to develop cavities. Consequently, these children are more likely to receive early and effective treatment when needed (9). Thus, parental education level can serve as a valuable predictor for assessing the incidence of dental caries in children. Conversely, there is a correlation suggesting that children from families with lower parental education levels tend to experience more cavities. These data are supported by the results of our study, which suggest that parents' level of education influences children's oral health. Although the statistical associations typically fall within expected ranges, they still meet the conventional statistical thresholds (p≤0.05). Thus, the present study revealed that children are more likely to adopt regular oral hygiene practices as parental education levels increase; in particular, children from families with parents with higher education practiced brushing their teeth more frequently. At the same time, these children, mostly guided by their parents, opted to use contemporary brushing methods, opting for electric brushes or com

Table 2. Impact of parental gender, education level, and socio-economic factors on oral hygiene – correlations based on Spearman's rank coefficient.

INDICATORS	Cohort	Education level	Living environment	Income	Visit with children	Frequency of brushing	Brush type	Brush change frequency	Type of brushing	Brushing time	Flossing	Tongue brushing
Parental gender	G	-0.01	-0.03	-0.06	-0.09	-0.07	0.01	-0.02	0.04	0.04	0.01	-0.06
	R	-0.01	0	-0.11	-0.09	-0.06	-0.01	-0.07	0.11	0.07	0.05	-0.07
	U	-0.01	0	0	-0.06	-0.07	0.03	0.12	-0.03	0.03	-0.01	-0.06
Education level	G		0.41	0.35	0.29	0.24	0.27	-0.13	0.18	0.25	0.30	0.21
	R		0	0.30	0.26	0.16	0.13	-0.28	0.13	0.23	0.22	0.19
	U		0	0.34	0.14	0.20	0.25	0.01	0.12	0.12	0.12	0.06
Living environment	G	0.41		0.16	0.29	0.20	0.22	-0.05	0.15	0.22	0.37	0.23
Income	G	0.35	0.16		0.12	0.17	0.15	-0.06	0.05	0.16	0.23	0.15
	R	0.30	0		0.13	0.16	0.04	-0.03	0.03	0.08	020	0.15
	U	0.34	0		-0.01	0.11	0.18	-0.06	0.02	0.16	0.17	0.08
Visit with children	G	0.29	0.29	0.12		0.23	0.12	-0.22	0.29	0.16	0.22	0.23
	R	0.26	0	0.13		0.24	0.02	-0.27	0.30	0.09	0.14	0.20
	U	0.14	0	-0.01		0.08	0.12	-0.13	0.21	0.15	0.12	0.12
Frequency of brushing	G	0.24	0.20	0.17	0.23		0.09	-0.17	0.21	0.23	0.20	0.25
	R	0.16	0	0.16	0.24		-0.05	-0.15	0.23	0.16	0.08	0.21
	U	0.20	0	0.11	0.08		0.10	-0.17	0.14	0.23	0.18	0.21
Brush type	G	0.27	0.22	0.15	0.12	0.09		-0.04	0.08	0.27	0.22	0.16
	R	0.13	0	0.04	0.02	-0.05		-0.09	0.01	0.26	0.22	0.18
	U	0.25	0	0.18	0.12	0.10		0	0.1	0.22	0.12	0.1
Brush change frequency	G	-0.13	-0.05	-0.06	-0.22	-0.17	-0.04		-0.23	-0.16	-0.07	-0.23
	R	-0.28	0	-0.03	-0.27	-0.15	-0.09		-0.28	-0.21	-0.05	-0.29
	U	0.01	0	-0.06	-0.13	-0.17	0		-0.18	-0.1	-0.08	-0.17
Type of brushing	G	0.18	0.15	0.05	0.29	0.21	0.08	-0.23		0.22	0.18	0.24
	R	0.13	0	0.03	0.30	0.23	0.01	-0.28		0.28	0.16	0.30
	U	0.12	0	0.02	0.21	0.14	0.09	-0.18		0.12	0.10	0.15
Brushing time	G	0.25	0.22	0.16	0.16	0.23	0.27	-0.16	0.22		0.24	0.29
	R	0.23	0	0.08	0.09	0.16	0.26	-0.21	0.28		0.13	0.26
	U	0.12	0	0.16	0.15	0.23	0.22	-0.10	0.12		0.19	0.26

Flossing	G	0.30	0.37	0.23	0.22	0.20	0.22	-0.07	0.18	0.24		0.26
	R	0.20	0	0.20	0.14	0.08	0.22	-0.05	0.16	0.13		0.17
	U	0.12	0	0.17	0.12	0.18	0.12	-0.08	0.10	0.19		0.22
Tongue brushing	G	0.21	0.23	0.15	0.23	0.25	0.16	-0.23	0.24	0.29	0.26	
	R	0.19	0	0.15	0.20	0.21	0.18	-0.29	0.30	0.26	0.17	
	U	0.06	0	0.08	0.12	0.21	0.10	-0.17	0.15	0.26	0.22	

Notes: results (r_s) selected with **Bold** registered $p \leq 0.05$,
G – general cohort,
R – rural,
U – urban.

bining them with manual ones. Parental education also positively impacts the frequency of toothbrush replacement, which contributes to better brushing quality through various brushing techniques and increased time spent on oral care. Furthermore, children from these families prove awareness on the importance of tongue brushing.

Parental education also influences the higher prevalence of children who, in addition to brushing, also use other hygiene practices. Thus, dental floss and mouthwash have become essential tools for children whose parents are well-educated. According to Chen et al. (2020), this phenomenon can be explained by educated parents possessing greater knowledge in the field of oral health, and who have more demands on the quality of the dental service, as well as learn and check their children's oral hygiene skills more rigorously (2).

Moreover, highly educated parents not only understand the benefits of preventive methods but also have an analytical thinking, reflected in thoughtful decisions regarding healthcare facilities (location, distance), the components used, and their characteristics, paying less attention to children's complaints.

Among the socioeconomic factors influencing oral hygiene, Tas et al. (2017) identified parents' educational level as the most significant, surpassing family income and occupational status (10). A higher level of maternal education was positively correlated with a lower incidence of minor caries. At the same time, lower levels of paternal education, family income, and occupational status (employed/unemployed) were associated with more severe cases of dental caries. Walker et al. (2017) studied 130 respondents and found that mothers, compared to fathers, are generally more attentive to their children's diet and hygiene practices, including the frequency and quality of brushing (11). Branden et al. (2013) also found that chil-

dren of more educated mothers consumed fewer sugary drinks, brushed their teeth more frequently, and visited the dentist regularly for preventive care, resulting in a lower incidence of dental caries (12). To examine potential differences in the education process based on the gender of the parent, the study found that mothers more frequently visited the dentist with their children. Additionally, mothers were more attentive to the frequency of changing toothbrushes, the brushing techniques used, flossing habits, and the use of mouthwash (tab. 1).

Clinical Significance. The study results highlight several concerning issues, including existing social inequalities in dental pathologies, emphasizing the need for preventive strategies. The findings revealed an uneven distribution of oral health habits across different social strata, which can be used towards the development of targeted national strategies.

The study has several limitations. According to Gurav et al. (2022), prophylactic interventions such as classes, games, and animations have proven effective in improving oral hygiene

and reducing caries. However, these interventions did not reduce plaque formation and gingival inflammation compared to the group having regular dental checkups.

Therefore, the results do not refer to the number of dental visits per year or the reasons for attending the dentist. Additionally, no data were collected on the severity of the injuries or the thera-peutic or prophylactic procedures performed. Another limitation was related to the degree of correlation, which, although mostly statistically significant within the 95% range, showed a very weak association. However, the arguments were supported by the regression results. Although various factors contribute to children's oral hygiene, the educational level of parents should be regarded as fundamental.

CONCLUSIONS

1. Children's oral hygiene skills are improved by socio-economic factors as well as the attitude and care parents show towards them.

2. Raising the parental education level, whether from urban or rural areas, positively influences the development of proper hygiene habits in children, thus ensuring oral health in adulthood. Educated parents positively influence various aspects of a child's oral hygiene at home, including the number of brushings per day, the type of toothbrush used, the frequency of replacing brushes, brushing techniques, tongue cleaning, and the use of dental floss and mouthwash. Additionally, higher education levels lead parents to visit the dentist more often, thus supporting the promotion of oral hygiene courses in school curriculum.

3. Prioritizing effective oral health literacy programs is essential for both the educational community and society as a whole.

CONFLICT OF INTERESTS

The authors declare no conflict of interest regarding this article.

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There is no information.

ETHICAL APPROVAL

The design of study was discussed and approved in the Scientific Forum Report no.10 of 25.05.2023, according to the master's higher studies program, cycle II in Public Health Management, within the School of Public Health, *Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova.

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COMPOSITION OF INDICATOR BACTERIA IN INDUSTRIAL AND BACKYARD CHICKEN FARMING

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Keywords: microflora, chicken, zoonosis, housing conditions.

Introduction. *The intestinal microbiocenosis is the most complex and important biotope of the body formed in the process of individual development.*
Material and methods. *The study was conducted on groups of 20-25-day-old chicks. The first group was housed under standard vivarium conditions with artificially maintained optimal climatic parameters. The second group was raised in a rural homestead in the Kyiv region, on pasture with access to water, and fed twice daily with a blend of grains supplemented with kitchen wastes. Samples of chicken droppings (10 per group) were analyzed according to current international ISO standards using certified nutrient media and equipment.*
Results. *Escherichia coli, Klebsiella spp., Enterococcus spp. were isolated from 100% of samples from chickens kept in simulated conditions of an industrial poultry house, and Pseudomonas aeruginosa was isolated from 70% of samples. E. coli and Enterococcus spp. were isolated from free-range chickens in 100% of cases. The analysis revealed that in pasture-raised chickens, Klebsiella spp. and P. aeruginosa were absent from the litter, with significantly higher levels of normal microflora (Enterococcus spp.).*
Conclusions. *Backyard-raised chickens showed no pathogenic zoonotic bacteria, in contrast to those raised under controlled conditions with optimal climate and standard diets.*

Cuvinte-cheie: microfloră, pui, zoonoze, condiții de trai.

RAPORTUL INDICATORILOR BACTERIENI LA PUII CRESCUȚI ÎN CONDIȚII INDUSTRIALE ȘI LA PUII CRESCUȚI ÎN AER LIBER

Introducere. *Microbiocenoza intestinală, formată în procesul dezvoltării individuale, reprezintă cel mai complex și cel mai important biotop al organismului.*
Material și metode. *Au fost formate două loturi de studiu, constituite din pui de 20-25 de zile. Primul grup a fost ținut în condiții standard de vivarium, cu asigurarea artificială a condițiilor climatice optime. Al doilea grup a fost ținut într-o gospodărie sătească din regiunea Kiev, pe pășune, având acces liber la apă și la hrană (un amestec de cereale cu adaos de deșeuri de bucătărie) de două ori pe zi . Probele de excremente de pui (10 per grup) au fost analizate în conformitate cu standardele internaționale ISO actuale, folosind medii și echipamente nutritive certificate.*
Rezultate. *În probele recoltate de la puii care au fost ținuți în condiții simulate, într-un adăpost industrial de păsări, în proporție de 100% au fost izolate Escherichia coli, Klebsiella spp. și Enterococcus spp., iar Pseudomonas aeruginosa a fost identificată în 70% de probe. La puii crescuți în aer liber, E. coli și Enterococcus spp. au fost izolate, de asemenea, în 100% dintre cazuri. Analiza rezultatelor a arătat că în așternutul puilor ținuți „pe pășune" nu s-au depistat Klebsiella spp. și P. aeruginosa, determinându-se, în același timp, un conținut semnificativ mai mare de reprezentanți ai microflorei normale (Enterococcus spp.).*
Concluzii. *La găinile crescute în condiții de curte s-a constatat absența bacteriilor zoonotice cu potențial patogen, spre deosebire de puii ținuți în condiții climatice optime, asigurate artificial, cu o dietă standard.*

INTRODUCTION

The gut microbiome plays a crucial role in various aspects of chicken physiology, including growth, feed conversion efficiency, immune system development, homeostasis maintenance, metabolic regulation, and resistance to pathogens (1, 2, 3). Recent studies have highlighted the significant impact of environmental factors on the gut microbiota (4).

During the formation of eggs in the oviduct and their passage through the reproductive tract, bacterial contamination can occur. Moreover, the embryonic stage of chickens already harbors diverse microorganisms within their digestive tracts (5). The environment of incubation cabinets significantly affects the formation of the microbiocenosis of the digestive tract of chickens. Furthermore, microbial contamination of eggshells serves as a potential source of bacteria for chickens, often occurring immediately after laying due to contact with contaminated processing equipment (6, 7). The microbial composition introduced into the body post-hatching varies depending on numerous factors, including production practices, husbandry technologies, feeding systems, etc. (8).

From the moment chickens hatch, their digestive tract becomes populated by a variety of environmental microorganisms such as *E. coli*, bacteria from genera like *Lactobacillus, Bacillus, Streptococcus, Bifidobacterium*, etc. (9). The composition of this intestinal microbiota is influenced by numerous factors, including diet, climate, and environmental conditions (10, 11), as well as other factors (12, 13). Considering the above, it is important to study how the housing environment impacts the species composition of poultry microbiota to optimize biosecurity protocols.

Microbial populations vary significantly across different segments of birds' digestive systems. For instance, concentrations range from 10^3-10^4 CFU/g in the stomach, 10^2-10^3 CFU/g in the glandular and muscular stomachs, and 10^3 CFU/g in the duodenum. The most microorganisms are found in the end sections of the small intestines, cecum, and rectum, where it ranges from 10^7 to 10^9 CFU/g, respectively.

The findings from recent research (14, 15) suggest that *Lactobacillus* spp., *Bifidobacterium* spp., *Klebsiella pneumoniae, Klebsiella ozaenae, Escherichia coli, Escherichia fergusonii, Enterobacter aerogenes, Eubacterium* spp., *Pseudomonas aeruginosa, Micrococcus luteus, Staphylococcus lentus*, and *Sarcina* spp. are indicative of the body's homeostasis in specific regions of the digestive tract.

Lactobacillus spp. typically colonize the digestive tract (16, 17), inhabiting various regions from the oral cavity to the rectum. These bacteria produce lactase, lysozyme, lactic acid, hydrogen peroxide, and various antibiotic-like compounds (such as lactocidin, lactocin, reuterin, plantaricin, lactolin, acidophilin) that inhibit the growth of putrefactive opportunistic microbes and pathogens causing acute intestinal infections. Upon interacting with enterocytes, they stimulate the bird's defense mechanisms, maintain colon acidity at pH 5.5-5.6, stimulate the phagocytic activity in neutrophils and macrophages, promote immunoglobulin synthesis and interferon formation, and participate in proteolysis and lactose metabolism processes.

Analysis of cecal microbiota using molecular approaches has identified bacterial populations of more than 600 species from more than 100 genera. However, many of these bacteria remain unclassified species or genera (18, 19). Previous research (20) established gram-positive cocci, *Clostridium* spp., *E. coli, Lactobacillus* spp., *Streptococcus* spp., *Acinetobacter* and *Acidobacteri*a which dominate the microbiota of the small intestine, while *Bacteroides,* whereas *Bacteroides* and *Clostridium* predominate in the cecum (21).

Maintaining a healthy gut is intricately linked to a balanced interaction between the immune system and the endogenous microbiota (22). A healthy avian intestine, as a rule, participates in the maintenance of intestinal homeostasis with the help of a complex network of cells and their secreted soluble products (23). The intestinal microbiota plays a crucial role in modulating the host's immune system, influencing organ development, and regulating host metabolism (24). Mucosal immune responses to resident intestinal microbiota can distinguish commensal from pathogenic bacteria (25). Gut microbiota is also involved in modulating B-cell response and immunoglobulin A (IgA) production. IgA plays an important role in regulating the composition of the intestinal microbiota through specific binding to bacterial epitopes. Thus, according to Aruwa C.E. et al. (26), maintaining intestinal health is para-

mount for the efficient and sustainable functioning of the avian digestive tract.

This study aimed to investigate the species composition of indicator opportunistic bacteria in the droppings of clinically healthy chickens that were kept under different environmental conditions.

MATERIAL AND METHODS

The study was carried out on groups of 20-25-day-old chicks, which were kept in different conditions. The first group of chickens (cross Cobb 500) was raised within vivarium conditions in NUBIP of Ukraine, housed in a standard KR 108 collapsible cage designed for laying hens and broilers that received a standard artificial diet under optimal climatic conditions (30±3°C, humidity 55±5%).

The second group of chicks was raised in a homestead located in the village of Gatne, Fastiv district, Kyiv region, within a free-range setting, having unrestricted access to water, being fed a diet of home-grown wheat and corn, which were locally sourced, chopped, steamed, and supplemented with kitchen wastes.

Throughout the study, all protocols adhered strictly to the guidelines outlined in EU Directive 2010/63/EU concerning the ethical treatment of animals used for scientific research.

Samples of chicken droppings (10 samples per group) from clinically healthy chickens were delivered in a thermal container at a temperature of 2-8°C. The research was conducted at the scientific laboratory of the Faculty of Veterinary Medicine using certified nutrient media and equipment, in accordance with the following regulatory standards.

Preparation of test samples, initial suspension and tenfold dilutions for microbiological examination was carried out in accordance with ISO 6887-1:2017 "Microbiology of the food chain Preparation of test samples, initial suspension and decimal dilutions for microbiological examination Part 1: General rules for the preparation of the initial suspension and decimal dilutions".

Isolation and determination of the most probable number (MPN) of enterobacteria, *E. coli, Klebsiella* spp., were carried out in accordance with ISO 21528-1:2017: Microbiology of the food chain – Horizontal method for the detection and

enumeration of *Enterobacteriaceae* – Part 1: Detection of *Enterobacteriaceae"*.

Isolation and determination of the most probable number (MPN) of enterococci was carried out in accordance with DSTU 8534:2015 "Food products. A method for detecting and determining the number of enterococci".

The technique of the most likely number (MPN) involves the use of the MPN table with a 95% confidence interval and the corresponding formula for calculating the number of microorganisms.

Isolation and determination of *P. aeruginosa* was carried out in accordance with the "Methodological recommendations. Detection and identification of *P. aeruginosa* in environmental objects (food products, water, wastewater)".

Isolation and identification of *Salmonella* spp. carried out in accordance with ISO 6579-1:2017 "Microbiology of the food chain horizontal method for the detection, enumeration and serotyping of *Salmonella* part 1: detection of *Salmonella* spp."*.*

Isolation and identification of *Listeria* spp./*Listeria monocytogenes* was carried out in accordance with ISO 11290-1:2017 "Microbiology of the food chain Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. Part 1: Detection method".

Isolation and identification of *Yersinia enterocolitica* was carried out in accordance with ISO 10273:2017 "Microbiology of the food chain Horizontal method for the detection of pathogenic *Yersinia enterocolitica*".

RESULTS

Based on bacteriological studies of samples obtained from chicken groups 1 and 2, no *Yersinia* spp., *Salmonella* spp., *or Listeria* spp./*Listeria monocytogenes* were isolated.

In group 2 samples, *Enterococcus* spp. *and E. coli* were isolated in 10 cultures each, while *Klebsiella* spp. and *P. aeruginosa* were not found in chicken litter samples from this group.

Isolated cultures of *E. coli* are gram-negative motile rods, catalase-positive, oxidase-negative, facultative anaerobes that ferment glucose and lactose with the formation of acid and gas. In the liquid medium of meat peptone broth, daily cultures of *E. coli* formed a uniform turbidity with a small amount of white amorphous sediment that easily

broke up upon agitation.

On dense meat peptone agar (MPA), *E. coli* cultures formed S-shaped colonies measuring 2-4 mm in diameter, appearing transparent, delicate, and grayish in color. On XLD agar (xylose-lysine deoxycholate agar) (HiMedia), *E. coli* colonies exhibited a yellow coloration, with the surrounding medium changing from red to yellow. Additionally, *E. coli* cultures formed blue colonies on Salmonella differential agar (M1078 Raj Hansa medium) (HiMedia).

E. coli cultures did not grow on the selective differential diagnostic medium bismuth-sulfite agar M1031 (HiMedia). On the chromogenic medium HiCrome *E. coli* Agar M 12951 (HiMedia), isolated *E. coli* cultures formed distinct green colonies. *E. coli* cultures fermented glucose and lactose, producing acid and gas; nitrates were not reduced to nitrites, H_2S was not released; did not form urea; but did produce indole.

Cultures of *Klebsiella* spp. are gram-negative non-motile rods that form capsules. 16 – hourly colonies of *Klebsiella* spp. on solid nutrient media – dome-shaped, 3-4 mm in diameter, with a mucous membrane, on meat peptone agar (MPA) they displayed a grayish-white color; on the Endo medium, the colonies had a pale pink color; on XLD medium – colorless opaque colonies with yellow staining of the medium around the colonies. On HiCrome *E. coli* Agar M 12951 chromogenic medium, selected *Klebsiella* spp. cultures formed transparent, colorless colonies. *Klebsiella* spp. in MPB formed a uniform turbidity of the medium with a stretchy mucous sediment and a film on the surface of the broth culture. They fermented glucose, sucrose, and lactose; did not produce indole or hydrogen sulfide; reduced nitrates to nitrites; exhibited urease activity; and tested positive for lysine and negative for ornithine and phenylalanine.

20 *Enterococcus* spp. cultures were isolated during the studies of the material droppings samples. These gram-positive cocci or ovoids were facultative anaerobes, catalase-negative, and oxidase-negative. They fermented glucose to produce acid without gas, hydrolyzed esculin, and lacked hemolytic activity.

Isolated cultures of *P. aeruginosa* are small homogeneous gram-negative rods (ovoids in appearance, sized 1-5 x 0.5-1.0 microns). They exhibit a consistent turbidity in tryptone-soy broth. On ce-trimide agar, they form homogeneous yellow-green, small S-shaped colonies. These bacteria do not produce hydrogen sulfide, do not reduce nitrates, and do not ferment lactose or sucrose. They are capable of producing pyocyanin and demonstrate β-hemolytic activity.

The MPN of isolated groups of microorganisms was determined. In the first experimental group (tab. 1) *E. coli*, *Klebsiella* spp., *Enterococcus* spp. isolated from 10 chickens, whereas *P. aeruginosa* – from 7 chickens.

The MPN index for *E. coli* in chickens of the first group (tab. 1) ranged between 4.6×10^5–$> 1.1 \times 10^7$ colony-forming units (CFU) per gram of sample, with actual counts ranging from 9.0×10^4 to 4.0×10^7 CFU/g at a 95% confidence level. The average MPN index for *E. coli* in the samples was 1.0×10^7 CFU/g.

For *Klebsiella* spp., the MPN index in the first experimental group (tab. 1) ranged from 2.4×10^5 to 1.1×10^7 CFU/g, with actual counts ranging from 4.0×10^4 to 4.0×10^7 CFU/g at a 95% confidence level. The average MPN index for *Klebsiella* spp. in the samples of chicken droppings was 8.0×10^6 CFU/g.

The MPN index for *Enterococcus* spp. (tab. 2) in the chicken droppings from the first group ranged from 2.4×10^2 to 1.1×10^5 CFU/g, with actual counts ranging from 4.0×10 to 4.0×10^5 CFU/g at a 95% confidence level. The average MPN index for *Enterococcus* spp. in the samples was 1.8×10^4 CFU/g.

In the case of *P. aeruginosa* (tab. 2), the MPN index in the litter samples from chickens of the first group ranged from 4.6×10^3 to 1.1×10^7 CFU/g, with actual counts ranging from 9.0×10^2 to 4.0×10^7 CFU/g at a 95% confidence level. The average MPN index for *P. aeruginosa* was 1.6×10^6 CFU/g.

The analysis of the obtained data in Tables 1 and 2 showed that the chicken droppings samples differed in terms of species composition of isolated indicator bacteria in chicken droppings (tab. 3). *P. aeruginosa* was isolated in 70% of the samples (samples 1, 4, 5, 6, 8, 9, 10). Given the pathogenic potential associated with this bacterium, the data were categorized into subgroups: A (samples without *P. aeruginosa*) and B (samples where this pathogen was isolated).

Thus, in the samples of subgroup A (samples No. 2, 3, 7), the values of MPN for *E. coli* in all samples

Table 1. Bacteriological examination results (according to the MPN indicator)
of chicken droppings samples from group 1.

Samples from chickens, no	Indexes			
	E. coli, [1]CFU		*Klebsiella* spp., [1] CFU	
	Availability *E.coli,* [2] MPN in 1.0 g	The actual number microorganisms per gram within the 95% confidence interval	Availability *Klebsiella* spp., [2] MPN in 1.0 g	The actual number microorganisms per gram within the 95% confidence interval
1	4.6×10^5	$9.0 \times 10^4 - 1.96 \times 10^6$	$>1.1 \times 10^7$	-
2	$>1.1 \times 10^7$	-	$>1.1 \times 10^7$	-
3	$>1.1 \times 10^7$	-	$>1.1 \times 10^7$	-
4	$>1.1 \times 10^7$	-	$>1.1 \times 10^7$	-
5	$>1.1 \times 10^7$	-	$>1.1 \times 10^7$	-
6	$>1.1 \times 10^7$	-	2.4×10^5	$4.0 \times 10^4 - 9.9 \times 10^5$
7	$>1.1 \times 10^7$	-	2.4×10^5	$4.0 \times 10^4 - 9.9 \times 10^5$
8	$>1.1 \times 10^7$	-	2.4×10^6	$4.0 \times 10^5 - 9.9 \times 10^6$
9	$>1.1 \times 10^7$	-	$>1.1 \times 10^7$	-
10	$>1.1 \times 10^7$	-	$>1.1 \times 10^7$	-
min-max	$4.6 \times 10^5 - >1.1 \times 10^7$	$9.0 \times 10^4 - >4.0 \times 10^7$	$2.4 \times 10^5 - >1.1 \times 10^7$	$4.0 \times 10^4 - >4.0 \times 10^7$
Average value	1.0×10^7	-	8.0×10^6	-

Notes: (here and further): [1] CFU – colony-forming units; [2] MPN – most probable number.

Table 2. Results of bacteriological examination (according to the MPN indicator)
of chicken litter samples from group 1.

Samples from chickens, No	Indexes			
	P. aeruginosa, [1] CFU		*Enterococcus* spp., [1] CFU	
	Availability *P. aeruginosa* [2] **MPN** in 1.0 g	The actual number microorganisms per gram within the 95% confidence interval	Availability *Enterococcus* spp. [2] **MPN** in 1.0 g	The actual number microorganisms per gram within the 95% confidence interval
1	1.5×10^4	$3.0 \times 10^3 - 3.8 \times 10^4$	4.6×10^3	$9.0 \times 10^2 - 1.96 \times 10^4$
2	-	-	2.4×10^2	40.0-990.0
3	-	-	1.1×10^4	$2.0 \times 10^3 - 4.0 \times 10^4$
4	1.1×10^5	$2.0 \times 10^4 - 4.0 \times 10^5$	1.1×10^5	$2.0 \times 10^4 - 4.0 \times 10^5$
5	$>1.1 \times 10^7$	-	1.1×10^4	$2.0 \times 10^3 - 4.0 \times 10^4$
6	2.4×10^4	$4.0 \times 10^3 - 9.9 \times 10^4$	2.4×10^4	$4.0 \times 10^3 - 9.9 \times 10^4$
7	-	-	4.6×10^3	$9.0 \times 10^2 - 1.96 \times 10^4$
8	1.1×10^5	$2.0 \times 10^4 - 4.0 \times 10^5$	1.1×10^4	$2.0 \times 10^3 - 4.0 \times 10^4$
9	4.6×10^3	$9.0 \times 10^2 - 1.96 \times 10^4$	1.1×10^3	200.0-4000.0
10	1.1×10^4	$2.0 \times 10^3 - 4.0 \times 10^4$	2.4×10^3	$4.0 \times 10^2 - 9.9 \times 10^3$
min-max	$4.6 \times 10^3 - >1.1 \times 10^7$	$9.0 \times 10^2 - >4.0 \times 10^7$	$2.4 \times 10^2 - 1.1 \times 10^5$	40.0-4.0$\times 10^5$
Average value	1.6×10^6	-	1.8×10^4	

were within $>1.1 \times 10^7$ CFU/g; indicator of MPN for *Enterococcus* spp. ranged between $2.4 \times 10^2 - 1.1 \times 10^4$ CFU/g, the average value of the indicator was 5.3×10^3 CFU/g; indicators of NL for *Klebsiella* spp. ranged within $2.4 \times 10^5 - >1.1 \times 10^7$ CFU/g, the average value of the MPN indicator for *Klebsiella* spp. was 7.4×10^6 CFU/g.

The content of the indicator bacteria in the sam-

ples of subgroup B was as follows: MPN indicator for *E. coli* ranged from >1.1x10^7 CFU/g, with an average of 9.5x10^6 CFU/g; the low-frequency indicator for *Klebsiella* spp. ranged from 2.4x10^5 to >1.1x10^7 CFU/g, averaging 8.2x10^6 CFU/g; MPN indicator for *Enterococcus* spp. ranged from 1.1x10^3 to 1.1x10^5 CFU/g, averaging 2.3x10^4 CFU/g; MPN indicator for *P. aeruginosa* ranged from 4.6x10^3 to >1.1x10^7 CFU/g, averaging 1.6x10^6 CFU/g (tab. 3).

Thus, among the indicator microorganisms, investigated in animal droppings subgroups 1-A and 1-B, *E. coli* exhibited the highest concentration, which was slightly higher in subgroup 1-A than in subgroup 1-B. No significant difference in *Klebsiella* spp. counts was observed between the chicken droppings samples of these subgroups. However, *Enterococcus* spp. counts in subgroup 1-B were consistently higher by 1 lg compared to subgroup 1-A.

Table 3. The content of indicator bacteria in the chicken droppings from group 1.

Indexes	Value	Result, [1] CFU/g	
		group 1 A, n=3	group 1 B, n=7
[2] MPN *E. coli,* [1] CFU/g	D	>1.1x10^7	>1.1x10^7
	M	>1.1x10^7	9.5x10^6
[2] MPN *Klebsiellas* spp., [1] CFU/g	D	2.4x10^5->1.1x10^7	2.4x10^5->1.1x10^7
	M	7.4x10^6	8.2x10^6
[2] MPN *Enterococcus* spp., [1] CFU/g	D	2.4x10^2-1.1x10^4	1.1x10^3-1.1x10^5
	M	5.3x10^3	2.3x10^4
[2] MPN *P. aeruginosa.,* [1] CFU/g	D	-	4.6x10^3->1.1x10^7
	M	-	1.6x10^6

Notes (here and further): D – the range of MPN values in the group; M is the average value of MPN in the group.

In bacteriological studies of group 2 chicken droppings from free-range settings (tab. 4), *Klebsiella* spp. and *P. aeruginosa* were not detected, while *E. coli* cultures were isolated in 100% of cases, along with *Enterococcus* spp.

The most probable number (MPN) for *E. coli* in the samples studied ranged from 4.6x10^2 to 4.6x10^6 CFU/g, with the actual count of microorganisms ranging from 9.0x10^1 to 1.96x10^7 CFU/g at the 95% confidence level. The average MPN value for *E. coli* was 1.4x10^6 CFU/g.

MPN indicator for *Enterococcus* spp. in samples of litter from chickens from 2 groups ranged from 1.1x10^4 to >1.1x10^9 CFU/g, with the actual count of microorganisms ranging from 2.0x10^3 to >1.1x10^9 CFU/g at the 95% confidence level. The average MPN value for *Enterococcus* spp. was 3.4x10^8 CFU/g (tab. 4).

In chickens from two groups, the MPN indicator for *Enterococcus* spp. and *E. coli* exceeded 90% of the studied samples: in one sample – by 1 lg (sample No. 9); in 6 samples – by 3 lg (samples No. 1, 2, 4, 6, 7, 8); in 2 samples – by 4 lg (samples No. 5, 10). One sample showed quantitative indicators within one titer (1.1-2.4x10^4 CFU/g).

The analysis of the obtained results (tab. 5) showed that, *E. coli, Klebsiellas* spp., *Enterococcus* spp. were isolated from 100% of the samples from chickens kept in simulated industrial poultry house conditions (the first group).

Additionally, *P. aeruginosa*, an insidious causative agent of diseases in both chickens and humans, was isolated from 70% of these samples. *E. coli* and *Enterococcus* spp. were isolated from free-range chickens in 100% of cases. It should be noted that the isolated cultures exhibited cultural, morphological, and biochemical characteristics typical of their species, with no phenotypic signs of dissociation. In group 1 chickens, the average values of MPN indicators were as follows: *E. coli* – 1.0x10^7 CFU/g; *Klebsiella* spp. – 8.0x10^6; *Enterococcus* spp. – 1.8x10 CFU/g; *P. aeruginosa* – 1.6x10^6 CFU/g, while in free-range chickens (group 2) the average value of MPN indicator for *E. coli* was 1.4x10^6 CFU/g (i.e. lower by 1 lg); the average value of the MPN indicator for *Enterococcus* spp. – 3.4x10^8 CFU/g (i.e. higher by 4 lg). Notably, in free-range chickens, no potentially pathogenic *Klebsiella* spp. or *P. aeruginosa* were found; however, there was a significantly higher presence of *Enterococcus* spp., which is part of the normal intestinal microflora of chickens.

Table 4. Results of bacteriological examination (according to the MPN indicator) of chicken droppings samples from group 2.

Samples from chickens, no	Indexes			
	E. coli, [1] CFU		*Enterococcus* spp., [1] CFU	
	Availability *E. coli* [2] MPN in 1.0 g	The actual number microorganisms per gram within the 95% confidence interval	Availability *Enterococcus* spp. [2] MPN in 1.0 g	The actual number microorganisms per gram within the 95% confidence interval
1	4.6×10^6	$9.0 \times 10^5 - 1.96 \times 10^7$	$>1.1 \times 10^9$	-
2	4.6×10^2	$9.0 \times 10^1 - 1.96 \times 10^3$	1.1×10^5	$2.0 \times 10^4 - 4.0 \times 10^5$
3	2.4×10^4	$4.0 \times 10^3 - 9.9 \times 10^4$	1.1×10^4	$2.0 \times 10^3 - 4.0 \times 10^4$
4	4.6×10^6	$9.0 \times 10^5 - 1.96 \times 10^7$	$>1.1 \times 10^9$	-
5	4.6×10^4	$9.0 \times 10^3 - 1.96 \times 10^5$	1.1×10^8	$2.0 \times 10^7 - 4.0 \times 10^8$
6	4.6×10^6	$9.0 \times 10^5 - 1.96 \times 10^7$	$>1.1 \times 10^9$	-
7	4.6×10^3	$9.0 \times 10^2 - 1.96 \times 10^4$	1.1×10^6	$2.0 \times 10^5 - 4.0 \times 10^6$
8	2.4×10^3	$4.0 \times 10^2 - 9.9 \times 10^4$	1.1×10^6	$2.0 \times 10^5 - 4.0 \times 10^6$
9	2.4×10^4	$4.0 \times 10^3 - 9.9 \times 10^4$	4.6×10^5	$9.0 \times 10^4 - 1.96 \times 10^6$
10	2.4×10^3	$4.0 \times 10^2 - 9.9 \times 10^3$	2.4×10^7	$4.0 \times 10^6 - 9.9 \times 10^9$
min-max	$4.6 \times 10^2 - 4.6 \times 10^6$	$9.0 \times 10^1 - 1.96 \times 10^7$	$1.1 \times 10^4 - >1.1 \times 10^9$	$2.0 \times 10^3 - >1.1 \times 10^9$
Average value	1.4×10^6	-	3.4×10^8	-

Table 5. The content of indicator bacteria in the droppings of chickens from both groups, 1 and 2.

Indexes	Value	Result, [1] CFU/g	
		group 1 A, n=10	group 1 A, n=10
[2] MPN *E. coli*, [1] CFU/g	D	$4.6 \times 10^5 - >1.1 \times 10^7$	$4.6 \times 10^2 - 4.6 \times 10^6$
	M	**1.0×10^7**	**1.4×10^6**
[2] MPN *Klebsiellas* spp., [1] CFU/g	D	$2.4 \times 10^5 - >1.1 \times 10^7$	-
	M	8.0×10^6	-
[2] MPN *Enterococcus* spp., [1] CFU/g	D	$2.4 \times 10^2 - 1.1 \times 10^5$	$2.0 \times 10^3 - >1.1 \times 10^9$
	M	**1.8×10^4**	**3.4×10^8**
[2] MPN *P. aeruginosa*, [1] CFU/g	D	$4.6 \times 10^3 - >1.1 \times 10^7$	-
	M	1.6×10^6	-

DISSCUTIONS

This study focused on identifying differences in the content of indicator microorganisms. According to current concepts of risk assessment for habitats and ecosystems, various biological entities, including microorganisms (viruses, bacteriophages, bacteria, fungi), helminth eggs, microscopic algae, and a range of protozoa, can be used as indicators. Currently, certain bacteria are used to assess the status of water bodies (27 – 31). The presence of indicator microorganisms in a specific ecological or biological niche may signal the presence of other pathogens (32 – 34). For example, the bacteriophage *Bacteroides fragilis* has been proposed as a potential indicator of human viruses in the environment.

Common coliforms, fecal coliforms, *E. coli*, and enterococci are more often used as indicators of anthropogenic pressure on sources of drinking water.

Additionally, assessing fecal contamination of surface and drinking water using groups of microorganisms is quite common., e.g. – *Escherichia*, *Citrobacter*, *Enterobacter.* The following should be identified among possible indicator bacteria: *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Mycobacterium tuberculosis*, *Streptococcus bovis*, *Naegleria fowleri*, *Legionella* spp., *Mycobacterium*

spp., *Aeromonas* spp., *Acanthamoeba* spp., *Staphylococcus* spp., *Legionella* spp., *Salmonella* spp., *Shigella* spp., *Clostridium* spp., *Legionella* spp., *Yersinia* spp., *Campylobacter* spp., *Listeria* spp., yeast, etc. (35).

Identifying these indicator microorganisms and determining their sources of spread is crucial for controlling the risks of disease outbreaks, particularly those of zoonotic origin. This goal can be achieved by determining the phenotypic characteristics of the microorganisms or their genetic markers.

In recent years, methods for quantitatively determining specific segments of DNA or RNA in the genome, such as PCR and next-generation sequencing (NGS), have been employed to identify genetic

markers (36 – 40). The source of contamination is identified based on the analysis of genetic sequences unique to both the specific microorganism and the host organism from which it originates.

Phenotypic characteristics of indicator microorganisms are assessed using traditional bacteriological methods. When performing these studies, the test for determining the most probable number of microorganisms (MPN, NCH) was used to detect not only the presence of certain genera of bacteria, but also to estimate their number (41). The results obtained regarding the presence of indicator bacteria in chicken litter under different housing conditions support the significant influence of the macroorganism habitat on the microbiome's species composition (42 – 47).

CONCLUSIONS

1. The research results revealed a difference in the composition of indicator bacteria in chicken droppings under various keeping conditions. Chickens raised in backyard settings showed no presence of zoonotic bacteria with pathogenic potential, compared to chickens kept under artificially controlled optimal climatic conditions and fed on a standard diet.

2. The obtained results provide the basis for an in-depth study of the microbiome in the digestive tract of chickens under different keeping conditions. Additionally, they aim to elucidate the mechanisms of microbiome formation and influence, with the goal of improving the technology for producing safe, high-quality poultry products in backyard farms and developing effective recommendations to ensure proper bioprotection levels for poultry maintained for personal consumption.

CONFLICT OF INTEREST

All authors declare no competing interests.

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REQUIREMENTS FOR AUTHORS

Rules of drafting

The manuscript (written in English and French) 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 1.0, 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** (will reflect the topicality and the general presentation of the problem studied, purpose and hypothesis of the study)
- **MATERIAL AND METHODS**
- **RESULTS**
- **DISCUSSIONS**
- **CONCLUSIONS**

- **CONFLICT OF INTERESTS**
- **ACKNOWLEDGEMENT** (optional)
- **ETHICAL APPROVAL** (specify the presence or absence of a positive opinion from the ethics committee: no, date, institution ad informed consent)
- **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 AMA 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 si-milarly as in the first citation. The number of references should not exceed 50 sources. The scientific authors are responsible for the accuracy of their writings. The reference list should include only those references that have been consulted by the authors of the manuscript. The elements of the reference sources are written exactly in accordance with the requirements.

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CERINȚE PENTRU AUTORI

Reguli de tehnoredactare

Pregătirea manuscrisului (elaborat în limbile engleză și franceză) va fi în conformitate cu instrucțiunile publicate în: *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). Manuscrisele trebuie să fie cu font Cambria, dimensiune 11 puncte, spațiat la interval 1,0, aliniere justificată, câmpurile 2 cm pe toate laturile. Toate paginile trebuie să fie numerotate consecutiv (în colțul de jos, în partea dreaptă) și să includă nume-rotarea continuă a paginilor. Abrevierile trebuie să fie explicate la prima apariție în text și nu trebuie utilizate excesiv. Manuscrisele nu trebuie să depășească (fără a număra titlul, afilierea, rezumatul și refe-rințele): pentru articole de sinteză/referate – 4500 de cuvinte; pentru articole de cercetare – 3000 de cuvinte; pentru opinii ale experților – 2500 de cuvinte; prezentare de caz și imagini din practica clinică/laborator – 1700 de cuvinte; note experimentale și clinice – 1300 de cuvinte; recenzii și prezentări de carte – 2000 de cuvinte; articole didactice – 4000 de cuvinte. Volumul tabelelor și figurilor nu trebuie să depășească ⅓ din volumul manuscrisului. Revista își rezervă dreptul de a face orice alte modificări de formatare. Manuscrisele respinse nu sunt returnate.

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

Titlul și autorii

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

Structura manuscrisului

Manuscrisul va cuprinde următoarele subtitluri (scrise cu majuscule):

− **REZUMAT** (vezi cerințele mai jos)
− **INTRODUCERE** (se va reflecta actualitatea și prezentarea generală a problemei studiate, scopul și ipoteza studiului)

− **MATERIAL ȘI METODE**
− **REZULTATE**
− **DISCUȚII**
− **CONCLUZII**
− **CONFLICT DE INTERESE**
− **MULȚUMIRI ȘI FINANȚARE** (optional)
− **APROBAREA ETICĂ** (se va specifica prezența sau lipsa avizului pozitiv de la comitetul de etică: nr, data, instituția și acordul informat)
− **REFERINȚE**

Rezumatul va conține până la 1600 de semne cu spații și va cuprinde:

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

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

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

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

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

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EXIGENCES POUR LES AUTEURS

Normes de rédaction

La préparation des manuscrits (rédigés en anglais et français) 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 1,0, 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 vo-lume 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.lebrun@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** (reflétera l'actualité et la présentation générale du problème étudié, le but et l'hypothèse de l'étude)
- **METHODES**
- **RESULTATS**
- **DISCUSSIONS**
- **CONCLUSIONS**
- **CONFLIT D'INTERETS**
- **REMERCIEMENTS ET FINANCEMENT**
- **APPROBATION ÉTHIQUE** (préciser la présence ou l'absence d'avis favorable du comité d'éthique: no, date, institution et consentement éclairé)
- **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 *AMA*, 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 английском и французском 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 1,0, выравнивание по ширине, поля 2 см со всех сторон. Все страницы должны быть пронуме-рованы 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 названия, принадлеж-ности, резюме и литературы): для обзорных статей/рефератов – 4500 слов; для научных ста-тей – 3000 слов; для экспертных заключений – 2500 слов; для презентации случаев из клини-ческой/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 дос-таточно 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 инфор-мация – адрес электронной почты (*например:* контактная инфор-мация: 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** (будет отражать актуаль-ность и общее представление изучаемой проблемы, цель и гипотезу исследования)
- **МАТЕРИАЛЫ И МЕТОДЫ**
- **РЕЗУЛЬТАТЫ**
- **ДИСКУССИИ**
- **ВЫВОДЫ**
- **КОНФЛИКТ ИНТЕРЕСОВ**
- **БЛАГОДАРНОСТИ И ФИНАНСИРОВАНИЕ**
- **ЭТИЧЕСКОЕ ОДОБРЕНИЕ** (указать наличие или отсутствие одобрения со стороны комитета по этике: №, дата, учреждение и информирован-ное согласие)
- **ЛИТЕРАТУРА**

Резюме должно содержать 1600 знаков с пробе-лами и будет включать в себя следующие подзаго-ловки:

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

Резюме не должно включать таблицы, диаграммы и библиографические заметки, инфор-мацию, не представленную в исследовании.

Рисунки (графики, диаграммы). Текст, включен-ный в рисунки, должен быть написан в Cambria, размер 10 пунктов. Каждый рисунок должен со-провождаться 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. Каж-дая таблица должна сопровождаться заголовком. Они должны вставляться в текст, не превышая ши-рину страницы. Должны быть пронумерованы арабскими цифрами и указаны в тексте в скобках (*например:* таб. 1). Название таблицы должно рас-полагаться над таблицей в центре (*например:* Таб-лица 1).

Литература. Источники должны быть пронуме-рованы в порядке их появления в тексте. Ссылки на источники должны быть в стиле *AMA*, поме-щены в конце статьи и включать только источ-ники, цитируемые в тексте (упоминание номера источника в круглых скобках). Если один и тот же источник цитируется несколько раз, он будет пе-редан в тексте с тем же номером, что и первый раз. Общее количество источников не должно превы-шать 50. Ответственность за точ-ность данных ле-жит на авторе. Будут цитиро-ваться только те ис-точники, с которыми ознакомились авторы руко-писи. Компоненты справочных источников должны быть написаны строго в соответствии с требованиями.

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The *One Health* concept

Human health
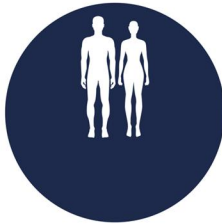
The WHO defined health in 1946 as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity", with the later addition of "the capacity to lead a socially and economically productive life".

Animal health

The OIE defines animal welfare in 2008: an animal is in good condition if it is healthy, enjoys comfort, is well fed, is safe, is able to display its innate (natural) behavior and does not suffer from unpleasant conditions such as pain, fear and stress.

Plant and environmental health

Environmental health refers to those aspects of human health that include the quality of life determined by physical, biological, socio-economic and psycho-social factors in the environment. The interrelationships of people with the environment concern medicine, when an ecological system is in a state of equilibrium, the health of the population prevails.

Globally, the *One Health* concept is a worldwide strategy to expand interdisciplinary collaborations and communications in all aspects related to the health care of humans, domestic animals or wildlife, which can no longer be approached separately, but only jointly.

One Health addresses not only human and animal disease concerns, but also issues related to lifestyle, diet, exercise, the impact of different types of human-animal relationships, and environmental exposures that can affect both populations. In order to achieve the expected effects, it is also necessary to educate the population to make them aware of the risk factors and benefits of prevention, as well as communication and understanding between patients and healthcare providers.