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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, founded 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.

One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems.

It recognizes that the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and interdependent.

While health, food, water, energy and environment are all wider topics with sector-specific concerns, the collaboration across sectors and disciplines contributes to protect health, address health challenges such as the emergence of infectious diseases, antimicrobial resistance, and food safety and promote the health and integrity of our ecosystems.

By linking humans, animals and the environment, One Health can help to address the full spectrum of disease control – from prevention to detection, preparedness, response and management – and contribute to global health security.

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Bridging science, policy and practice through One Health

Angela PARASCHIV, MD, PhD, Doctor Habilitatus, Associate Professor, MSc in International Public Health, Director, Nicolae Testemitanu School of Public Health, Nicolae Testemitanu State University of Medicine and Pharmacy, Republic of Moldova

Population health is increasingly shaped by processes that extend beyond the traditional boundaries of medicine and public health. Emerging and re-emerging infectious diseases, antimicrobial resistance, climate change, environmental degradation, food and water safety, population mobility, and other cross-border health threats demonstrate the profound interdependence between human health, animal health, and the ecosystems in which we live.

In this context, One Health is no longer merely an interdisciplinary concept; it has become an essential framework for research, education, public policy, and effective action. The complexity of contemporary health risks requires us to move beyond sectoral approaches and strengthen meaningful collaboration across public health, human and veterinary medicine, environmental sciences, agriculture, food safety, epidemiology, microbiology, social sciences, and public policy.

Risk management is an integral part of this approach. The capacity to identify threats at an early stage, assess risks using the best available evidence, integrate information across sectors and surveillance systems, and translate knowledge into timely decisions and interventions is fundamental to strengthening the resilience of health systems and societies.

From this perspective, the scientific journal One Health & Risk Management has an important role to play as a platform for scientific exchange and dialogue among researchers, academics, practitioners, and decision-makers. The publication and dissemination of high-quality research contribute not only to advancing scientific knowledge but also to bridging the gap between evidence, policy, and practice.

For the School of Public Health, the connection between education, research, policy, practice, and impact is fundamental. Universities and academic institutions have a responsibility not only to generate evidence, but also to prepare professionals capable of critically interpreting that evidence, assessing and communicating risks, and translating knowledge into effective solutions. At the same time, research must remain responsive to the real challenges faced by health systems and communities.

The Republic of Moldova has an important opportunity to further advance the One Health approach by strengthening interdisciplinary research, integrating One Health principles into education and professional development, enhancing capacities for risk assessment and management, and expanding national and international scientific cooperation. The European integration process provides an additional framework for alignment with international standards, participation in scientific networks, and the development of sustainable partnerships.

SYNTHESIS ARTICLE

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APOE4 AND CARDIOVASCULAR DISEASE: INTERPLAY OF DIETARY FATS IN MODULATING LDL LEVELS

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ABSTRACT

Introduction Apolipoprotein E (APOE) gene significantly affects lipid metabolism and CVD susceptibility, with its isoforms exerting different effects on cholesterol levels and cardiovascular health. Of particular concern is the APOE4 allele, which is linked to dyslipidemia, elevated LDL-C levels, and increased oxidative stress, thereby heightening the risk for coronary artery disease (CAD) and stroke. This review explores the complex relationship between APOE genotypes, lipid metabolism, and CVD risk, with a specific focus on the APOE4 allele.

Materials and methods This review followed a systematic literature review (SLR) methodology in accordance with PRISMA guidelines. Implementing a pre-defined eligibility and screening criteria, 48 studies were included in the review.

Results It highlights the influence of dietary fat intake on lipid profiles and the potential for targeted nutritional interventions in individuals carrying the APOE4 allele to mitigate genetic risk. Despite evidence linking APOE4 to impaired lipid clearance and cardiovascular pathology, research examining the interplay of genetics, nutrition, and metabolism in CVD remains limited.

Conclusions This paper emphasizes the importance of personalized dietary strategies tailored to an individual's APOE genotype to improve lipid metabolism, reduce CVD risk, and enhance overall cardiovascular health. By better understanding the genetic-nutritional relationship, clinicians and researchers can design more effective preventive and therapeutic strategies for individuals at higher genetic risk for CVD.

Keywords Cardiovascular Disease (CVD), Apolipoprotein E (APOE), LDL Cholesterol, Lipid Metabolism, APOE4 Genotype, Dietary Intervention.

APOE4 ȘI BOLILE CARDIOVASCULARE: INTERACȚIUNEA GRĂSIMILOR ALIMENTARE ÎN MODULAREA NIVELURILOR DE LDL

Introducere Gena apolipoproteinei E (APOE) influențează semnificativ metabolismul lipidic și susceptibilitatea la bolile cardiovasculare (BCV), iar izoformele sale exercită efecte diferite asupra nivelurilor de colesterol și asupra sănătății cardiovasculare. Un interes deosebit îl prezintă alela APOE4, asociată cu dislipidemia, creșterea nivelurilor de colesterol al lipoproteinelor cu densitate joasă (LDL-C) și intensificarea stresului oxidativ – factori care determină un risc crescut de boală coronariană (BC) și de accident vascular cerebral (AVC). Această revizuire analizează relația complexă dintre genotipurile APOE, metabolismul lipidic și riscul de BCV, cu accent deosebit asupra alelei APOE4.

Materiale și metode Lucrarea reprezintă o revizuire sistematică a literaturii (Systematic Literature Review – SLR), în conformitate cu recomandările PRISMA. Prin aplicarea unor criterii predefinite de eligibilitate și selecție, au fost incluse pentru analiză 48 de studii.

Rezultate Studiul relevă influența consumului de grăsimi alimentare asupra profilului lipidic și potențialul intervențiilor nutriționale țintite la purtătorii alelei APOE4 pentru atenuarea efectelor riscului genetic. Deși există dovezi care asociază APOE4 cu afectarea clearance-ului lipidic și dezvoltarea patologiei cardiovasculare, cercetările privind interacțiunea dintre factorii genetici, nutriție și metabolism în apariția bolilor cardiovasculare rămân incomplete.

Concluzii Lucrarea subliniază importanța strategiilor alimentare personalizate, adaptate genotipului APOE individual, pentru optimizarea metabolismului lipidic, reducerea riscului de boli cardiovasculare și îmbunătățirea sănătății cardiovasculare generale. O mai bună înțelegere a relației dintre factorii genetici și nutriționali ar putea facilita dezvoltarea de către clinicieni și cercetători a strategiilor preventive și terapeutice mai eficiente pentru persoanele cu predispoziție genetică la bolile cardiovasculare.

Cuvinte-cheie Boli cardiovasculare (BCV), apolipoproteina E (APOE), colesterol LDL, metabolism lipidic, genotip APOE4, intervenție nutrițională.

INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading cause of death worldwide, accounting for an accounting for an estimated 19.8 million fatalities globally (representing approximately 32% of all global deaths), with heart attacks and strokes responsible for 85% of these deaths (1). Over three-quarters of CVD-related deaths occur in low- and middle-income countries (1). Among premature deaths (under 70 years of age) attributed to non-communicable diseases, CVDs contribute to at least 38% (1). CVD refers to a group of conditions affecting the heart and blood vessels that include coronary artery disease (CAD), which can cause chest pain (angina) and heart attacks, as well as irregular heartbeats (arrhythmias), heart failure, and problems with heart valves. The development of atherosclerotic plaque in the coronary arteries results in inadequate blood and oxygen delivery to the myocardium and other regions of the body, posing it one of the most complex diseases with high morbidity and mortality worldwide (2). Cholesterol is widely recognized as a significant risk factor for atherosclerosis and cardiovascular diseases (CVD). Among the different forms of cholesterol, low-density lipoprotein cholesterol (LDL-C) is most closely linked to an elevated risk of coronary artery disease (CAD) and stroke. High levels of LDL-C contribute to the accumulation of cholesterol in arterial walls, resulting in plaque formation and vascular complications (3). The role of cholesterol in CVD is further influenced by dietary lipid intake, food choices, and overall lifestyle habits.

Research suggests that dietary components, such as specific fats, carbohydrates, fiber, antioxidants, and micronutrients, can influence lipid profiles and potentially be linked with CVD risk in APOE4 carriers. Body fat distribution is a better measure of disease risk than BMI (Body mass index), as central adiposity is associated with dyslipidemia and insulin resistance (4). Emerging evidence supports the integration of targeted dietary interventions, focusing on reducing LDL-C levels and improving lipid metabolism, to address the heightened susceptibility in this population (5,6). The literature indicates that preventing obesity and heart disease depends on diet, with the type of fat being more important than the amount. Dietary lipids enhance the taste, texture, and energy content of food while serving vital roles in the body, including providing energy, forming cell membranes, acting as precursors for signaling molecules, aiding the absorption of fat-soluble vitamins, and influencing cardiovascular health, as saturated fats raise LDL-C levels, whereas omega-6 polyunsaturated fatty acids lower total and LDL-C levels, with replacing saturated fats with omega-6 consistently shown to improve cholesterol profiles (7). Saturated fatty acids (SFA) found in common foods such as milk, butter, cheese, and various meats elevate LDL-C and high-density lipoprotein cholesterol (HDL-C). The rise in LDL-C occurs due to reduced hepatic clearance and increased production of LDL, a process associated with a decline in LDL receptor activity in the liver (5). Therefore, dietary fats play a potential role in lipid metabolism, which can contribute to the development of heart disease.

In addition to lifestyle factors, genetics plays a pivotal role in the risk of CVD and CAD. The Apolipoprotein E (APOE) gene, which encodes a 34 kDa glycoprotein composed of 299 amino acids primarily produced in the liver, is a critical player in lipid metabolism and disease pathophysiology (8). APOE is a ligand for LDL receptors, mediating the transport and clearance of cholesterol and triglyceride-rich lipoproteins. Its isoforms APOE2, APOE3, and APOE4exert varying effects on plasma lipoproteins and LDL levels, significantly influencing the risk of atherosclerosis and related conditions (9). The APOE4 allele is strongly associated with dyslipidemia, elevated LDL-C levels, and increased oxidative stress, making it a key genetic determinant of CAD and CVD risk. Studies indicate that carriers of the APOE4 allele have impaired lipid clearance, heightened oxidative stress, and disrupted lipid metabolism,

which collectively contribute to the pathogenesis of CAD (10,11). Furthermore, APOE4 is implicated in the onset of neuropathological lesions associated with Alzheimer's disease, highlighting its broad impact on lipid metabolism and systemic health (12,13).

Moreover, the APOE protein facilitates reverse cholesterol transport by forming high-density-like lipoproteins in macrophages, which are then transported to the liver. This function is critical for maintaining lipid homeostasis and underscores the significant influence of APOE on cardiovascular and systemic health (14). Advances in computational and bioinformatics studies further highlight the linear association of APOE4 with CAD, offering novel insights into the gene's role in disease progression and paving the way for future research (15-17). The interaction between the APOE4 genotype and dietary factors underscores the importance of personalized nutritional strategies to mitigate genetic risk.

The interplay between APOE4, lipid metabolism, and lifestyle factors presents an avenue for the development of targeted interventions. This study aims to systematically evaluate the interplay between the APOE4 genotype, dietary fat intake, and lipid metabolism in modulating LDL cholesterol levels and cardiovascular disease risk. Very limited and fragmented studies are present to elucidate and understand the genetic, nutritional, and metabolic relations in CAD which can guide for proper therapeutic development. By understanding the genetic underpinnings and nutritional modulations of APOE4-associated CVD risk, researchers and clinicians can design personalized approaches to reduce disease burden and improve outcomes in genetically susceptible populations. Further investigation into gene-nutrition interactions will be essential for advancing preventive strategies against CAD and CVD.

MATERIALS AND METHODS

Study Design

This review followed a systematic literature review (SLR) methodology to synthesize and critically appraise existing evidence on the relationship between the APOE4 genotype, lipid metabolism, dietary factors, and cardiovascular disease (CVD) outcomes. The study was conducted in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure methodological rigor and transparency.

Search Strategy

A comprehensive literature search (1990-2024) was conducted using the following electronic databases: PubMed, Scopus, and Web of Science. Searches were performed using a combination of Medical Subject Headings (MeSH) and free-text terms. The search terms included: "APOE4", "lipid metabolism", "dietary intervention", "LDL cholesterol", "cardiovascular disease", "dietary fats", "genetic polymorphism", and "lipoprotein particles".

Inclusion and Exclusion Criteria

The inclusion and exclusion criteria adopted for the selection of studies in this systematic review are summarized in Table 1.

Table 1. Defined Eligibility Criteria Guiding Study Inclusion and Exclusion in This Review

Criteria Type	Inclusion Criteria	Exclusion Criteria
Study Design	Peer-reviewed original research articles, systematic reviews, and meta-analyses	Editorials, letters, conference abstracts, and case reports
Language	Published in English	Published in non-English languages
Population	Human participants with reported APOE genotypes (specifically APOE4)	Animal studies, in vitro studies
Focus Area	Studies examining the link between APOE4, lipid metabolism, dietary intake, and cardiovascular outcomes	Studies focusing only on neurological outcomes (e.g., Alzheimer's disease)
Outcomes Reported	Includes at least one outcome related to LDL-C, HDL-C, VLDL, triglycerides, or CVD	No outcomes related to lipid metabolism or cardiovascular health
Intervention	Studies reporting dietary or pharmacological interventions relevant to lipid modulation in APOE4 carriers	Studies not discussing any dietary, genetic, or pharmacological intervention

Data Collection

Data collection for this systematic review was conducted through a structured screening and extraction process. After identifying relevant articles through comprehensive searches of PubMed, Scopus, and Web of Science, two independent reviewers screened all titles and abstracts for initial relevance. Full-text articles were then assessed based on the predefined inclusion and exclusion criteria. For each eligible study, key information was systematically extracted using a standardized data extraction form. The variables collected included study characteristics (authors, publication year, location), study design, sample size, participant demographics, APOE genotypes (particularly ε4 allele presence), lipid profile outcomes (such as LDL-C, HDL-C, VLDL, triglycerides), dietary interventions or patterns, and any pharmacological treatments evaluated. Additional data on cardiovascular outcomes, follow-up duration, and gene-diet interactions were also recorded where available. Any discrepancies between reviewers during data extraction were resolved through discussion or consultation with a third reviewer to ensure consistency and minimize bias.

Quality Assessment

The methodological quality of the included studies was evaluated using the QUADAS-2 tool across four domains. As shown Figure 1, most studies showed a low risk of bias in the index test domain due to validated lipid and genotyping methods. However, some concerns were noted in patient selection and reference standards, particularly in observational designs and studies using indirect measures. The flow and timing domain revealed the highest risk, often due to unclear follow-up durations and unreported attrition. Overall, most studies were rated as having some concerns, with a few showing high risk, highlighting the need for cautious interpretation of findings.

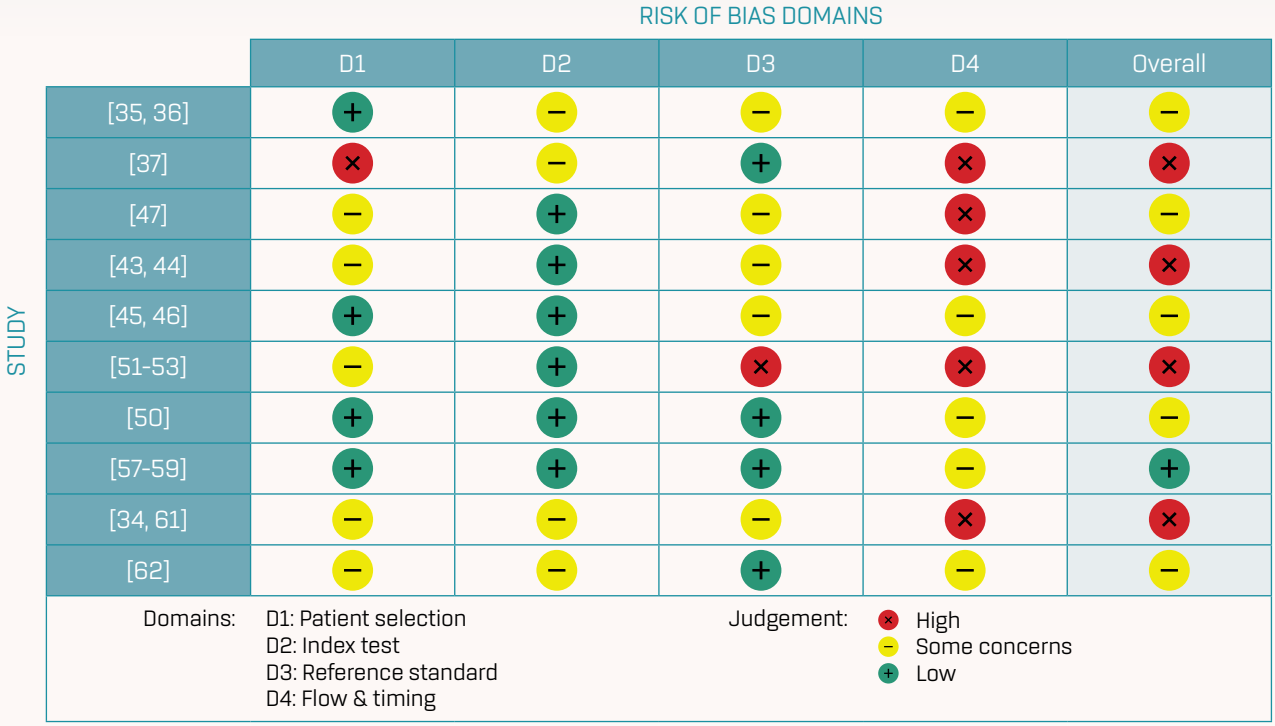

Figure 1. Risk of Bias Summary Based on QUADAS-2 Assessment.

RESULTS

Study Selection Process and Screening Flow

The comprehensive search strategy initially identified 1,186 records. After the removal of duplicates (n = 432), 754 articles were screened based on titles and abstracts. From these, 142 studies were selected for full-text review. Applying the predefined inclusion and exclusion criteria, a total of 48 studies were retained for the final synthesis. The detailed selection process is visually summarized in the PRISMA flow diagram, Figure 2.

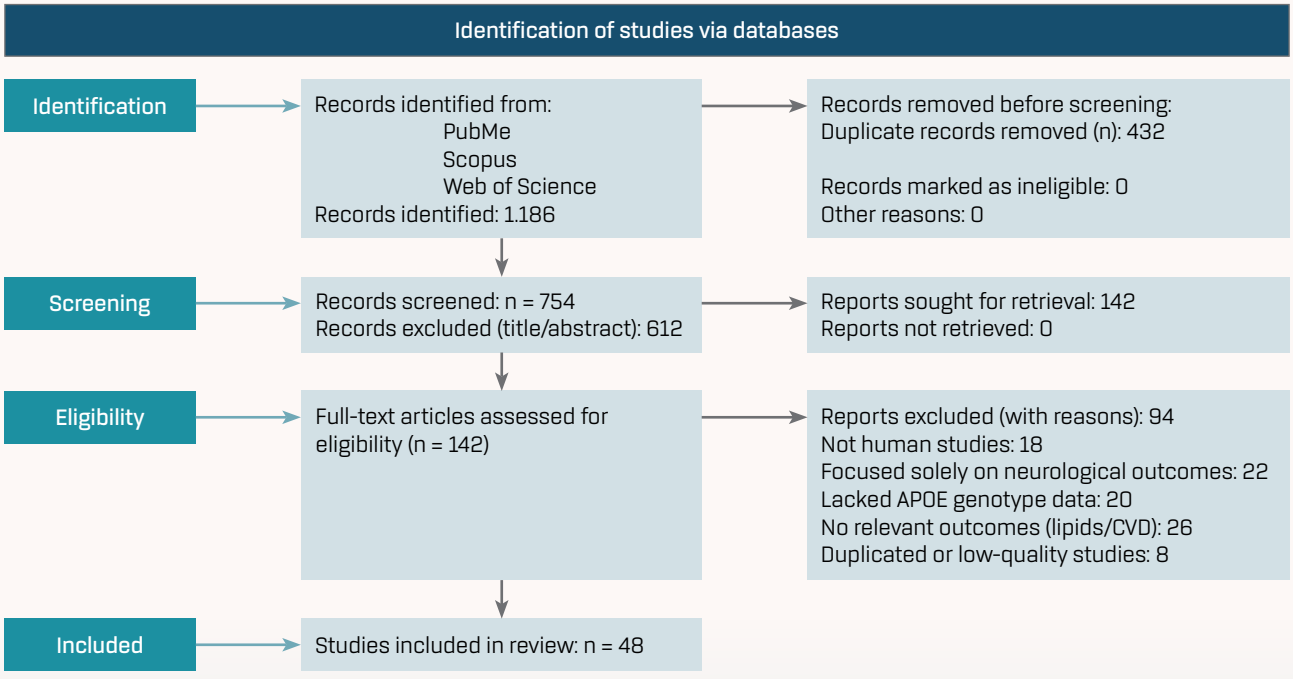

Figure 2. PRISMA 2020 Flow Diagram Illustrating the Systematic Study Selection Process.

Characteristics and Scope of Included Studies

The 48 studies included in this review were published between 1990 and 2024, encompassing research across a broad range of ethnically diverse populations from North America, Europe, Asia, and Latin America. Most studies focused on adult populations aged 18–75 years. Notably, approximately 70% of the included studies stratified outcomes based on APOE genotypes, with a particular emphasis on individuals carrying the ε4 allele.

To provide further insight into the research landscape, the year of publication of the included studies was analysed. The included studies cover a period of over 30 years, with a relatively low number of studies published before 2000, a slow increase in the early 2000s, and a significant rise after 2010. Peak numbers of studies were found from 2015 to 2024, suggesting a surge of interest in the effect of the interaction between APOE genotype and diet on cardiovascular risk. This reflects the growing awareness of the role of nutrigenomics and precision medicine in cardiovascular disease. Table 2 provides a comprehensive overview of the most relevant findings across multiple populations, linking APOE4 to lipid regulation and cardiovascular outcomes.

Table 2. Consolidated Evidence of APOE4-Linked Lipid Dysregulation, Dietary Interactions, and Cardiovascular Risk Across Populations.

Finding	Study/Population	Key Outcome
APOE4 associated with elevated LDL-C and TGs levels	Indian and Mexican populations (Das et al., 2008; Cahua-Pablo et al., 2022)	APOE4 linked to increased LDL-C and triglyceride concentrations in ethnically diverse populations
Preferential binding of APOE4 to VLDL leads to more efficient conversion into LDL	Gregg et al., 1986; Feingold, 2024	APOE4 enhances hepatic uptake of VLDL remnants and accelerates LDL production
Saturated fat intake increases LDL-C in APOE4 carriers	Sydney Diet Heart Study (1966–1973)	High SFA diets lead to adverse CVD outcomes in APOE4 individuals despite lowering total cholesterol
PUFA and MUFA intake reduce LDL-C, especially in APOE4 carriers	Observational and interventional dietary studies	Replacing SFA with PUFA/MUFA significantly improves lipid profiles
Trans fats increase LDL-C and decrease HDL-C, aggravating risk in APOE4 carriers	Meta-analyses and cohort studies	TFA intake linked to heightened cardiovascular risk due to its dual impact on LDL and HDL
Phytosterols lower LDL-C but may interfere with fat-soluble vitamins and have potential adverse effects	Animal and human studies	Plant sterols effectively reduce cholesterol but may have risks for red blood cell integrity and hormone regulation
Fiber reduces LDL-C by interrupting intestinal absorption and enhancing LDL receptor expression	Dietary intervention studies	Soluble and insoluble fiber intake improves lipid profiles and reduces CVD risk
Pharmacological agents like statins, ezetimibe, and PCSK9 inhibitors show varying efficacy in APOE4 carriers	Clinical trials and pharmacogenetic reviews	Statins are generally effective; PCSK9 inhibitors and ezetimibe provide additive benefits, especially where statin tolerance is low
APOE4 genotype modifies individual response to dietary and pharmacological interventions	Multiple studies in nutrigenomics	Highlights the importance of personalized nutrition and pharmacogenetics
Obesity exacerbates APOE4-mediated dyslipidemia by disrupting cholesterol absorption and insulin sensitivity	Epidemiological data and mechanistic studies	Visceral fat impacts lipid metabolism pathways, increasing CVD risk in APOE4 carriers

When it comes to study design, the evidence was a combination of observational, interventional and mechanistic studies. Most of the evidence came from observational studies (cross-sectional, case-control, and cohort designs) due to the novelty of the gene-diet interaction in population-based studies. A minority of the studies included randomized controlled trials (RCTs), which mainly involved dietary interventions and blood lipids. Moreover, a few mechanistic, experimental and pharmacogenetic studies were also included to understand the mechanisms of APOE4-related lipid metabolism. This mix suggests that although there is a large amount of associative evidence, there is relatively little high-quality randomized evidence (tab. 3).

Table 3. Distribution of Included Studies by Study Design.

Study Design Category	Description	Approximate Representation
Observational Studies	Cohort, case-control, cross-sectional	Majority
Randomized Controlled Trials (RCTs)	Dietary or pharmacological interventions	Limited
Mechanistic/Experimental Studies	Molecular, in vitro, or metabolic pathway studies	Moderate
Reviews/Meta-analyses (if included)	Secondary evidence synthesis	Minor

DISCUSSION

The findings of this review underscore the critical role of the APOE4 genotype in lipid metabolism, particularly its association with elevated LDL cholesterol levels and increased cardiovascular disease risk. Furthermore, the interplay between dietary fat intake and APOE4-mediated lipid regulation highlights the need for targeted nutritional interventions in genetically susceptible individuals.

Understanding the APOE Gene: Role in CVD

The APOE gene (3598 bp) maps to chromosome 19, and codes for APOE protein, a glycoprotein containing 299 amino acids of molecular weight 34 kDa, associated with lipid particles, that mainly functions in lipoprotein-mediated lipid transport between organs via the plasma and interstitial fluids (18). The protein involves three APOE variants (isoforms) in human lipid homeostasis and mediates the binding of VLDL and IDL to LDL-R and LDL receptor-related proteins (19,20). The single locus on chromosome 19 that produces three isoforms are APOE2, APOE3, and APOE4 from three alleles viz, epsilon 2 (ε2), epsilon 3 (ε3), and epsilon 4 (ε4) and the difference among them is due to amino acid substitution at positions 112 and 158 (21). Among the three, APOE3 is the most prevalent form which has cysteine residue at 112 and arginine at 158, whereas APOE4 has arginine at both 112 and 158 positions, and APOE2 has cysteines at both the sites, making them contribute significantly to coronary artery disease (CAD) and atherosclerosis (22) (tab. 4).

Table 4. Summary of the Differences among APOE Isoforms.

Differences	APOE4	APOE3	APOE2	References
Sequence Difference	112: Arg	112: Cys	112: Cys	(21,23-25)
	158: Arg	158: Arg	158: Cys	
Domain Interaction	Ionic interaction between Arg-61 and Glu-255	Absent	Absent	(26)
Binding Affinity	APOE4 binds to large LDL and VLDL	APOE3 and APOE2 bind to small HDL		(27, 28)
Prevalence (Allelic Frequency)	14%	78%	7%	(29, 30)
Disorders	CAD; CVD; AD	Normal	Type-III hyperlipoproteinemia	(31-33)

CAD = coronary artery disease; CVD = cardiovascular disease; AD = Alzheimer's disease.

However, not all studies demonstrate a consistent magnitude of effect between APOE4 and lipid levels, and in some cases, differences in LDL receptor affinity or plasma clearance rates are marginal. These discrepancies suggest that additional mechanisms beyond lipoprotein binding, such as hepatic lipid regulation or inflammatory pathways, may be more central to APOE4's atherogenicity.

The amino acid differences in the three APOE isoforms result in distinct structural properties that influence their functions, such as domain interaction and protein stability (molten globule formation). Studies have shown that replacing cysteine with arginine at position 158 in APOE2 significantly reduces its ability to bind to LDL receptors, while a similar substitution at position 112 in APOE3 does not affect its binding activity compared to APOE4 (25). The unique structure of APOE4 plays a key role in atherosclerosis, although the exact mechanism of how it interacts with LDL receptors and triggers structural changes remains unclear (32). Studies reported that the receptor-binding activity of APOE4 is thought to contribute to its association with a higher risk of CAD, linked to its role in cholesterol metabolism (15,17). Additionally, its unique domain interaction and protein stability further distinguish APOE4 from APOE3, contributing to its role in disease development. Literature has increasingly highlighted the APOE4 variant is strongly linked to higher risks of CVD, Alzheimer's disease, and cognitive decline, with individuals carrying one or two copies of the allele being more susceptible to these conditions (34).

APOE4 in Modulating LDL-C Levels

Several studies conducted across diverse populations have reported a correlation between the ApoE4 isoform and elevated lipid levels. For instance, Das et al., (2008) (35) observed increased levels of LDL-C and triglycerides (TG) in the Indian population, while Cahua-Pablo et al., (2022) (36) found similar trends among Mestizo women from Southwestern Mexico. These findings suggest a consistent association between the ApoE4 allele and dyslipidemia across different ethnic groups (35,36). However, these studies often lack stratified clinical outcomes or adjustments for confounding variables like obesity, physical activity, or co-medications, limiting their generalizability.

Though APOE4 is thought to promote more efficient conversion of VLDL remnants into LDL, some studies report minimal genotype-based differences in hepatic clearance rates. This variability may reflect population-specific factors, such as metabolic status or background diet, which are rarely controlled for uniformly (37-39).

With regards to lipid transport and atherosclerosis, studies discussed how cholesterol and triglycerides, being water-insoluble, are transported in the blood via lipoproteins such as LDL, HDL, VLDL, with some promoting plaque buildup in arteries (pro-atherogenic) and others helping to remove cholesterol (anti-atherogenic). The body uses two main pathways – the exogenous (from dietary fats) and endogenous (from the liver) – to move these lipids around. HDL, in particular, plays a protective role by helping remove excess cholesterol from tissues and transporting it back to the liver, a process that helps prevent atherosclerosis (40). Another study assigned participants to a dietary intervention group (n=221) and a control group. The intervention, which increased the intake of triglyceride-rich lipoproteins such as chylomicrons and VLDL, resulted in a higher concentration of ApoE per lipoprotein particle. This elevated ApoE per TAG-rich lipoprotein may enhance competition with LDL for LDL receptor-mediated clearance, potentially leading to increased circulating LDL-C levels (41).

As illustrated in Figure 3, the ApoE4-mediated pathway of VLDL metabolism demonstrates a preferential binding affinity of ApoE4 to VLDL particles, which influences their hepatic processing and subsequent conversion into IDL and LDL, thereby impacting plasma LDL-C concentrations [5].

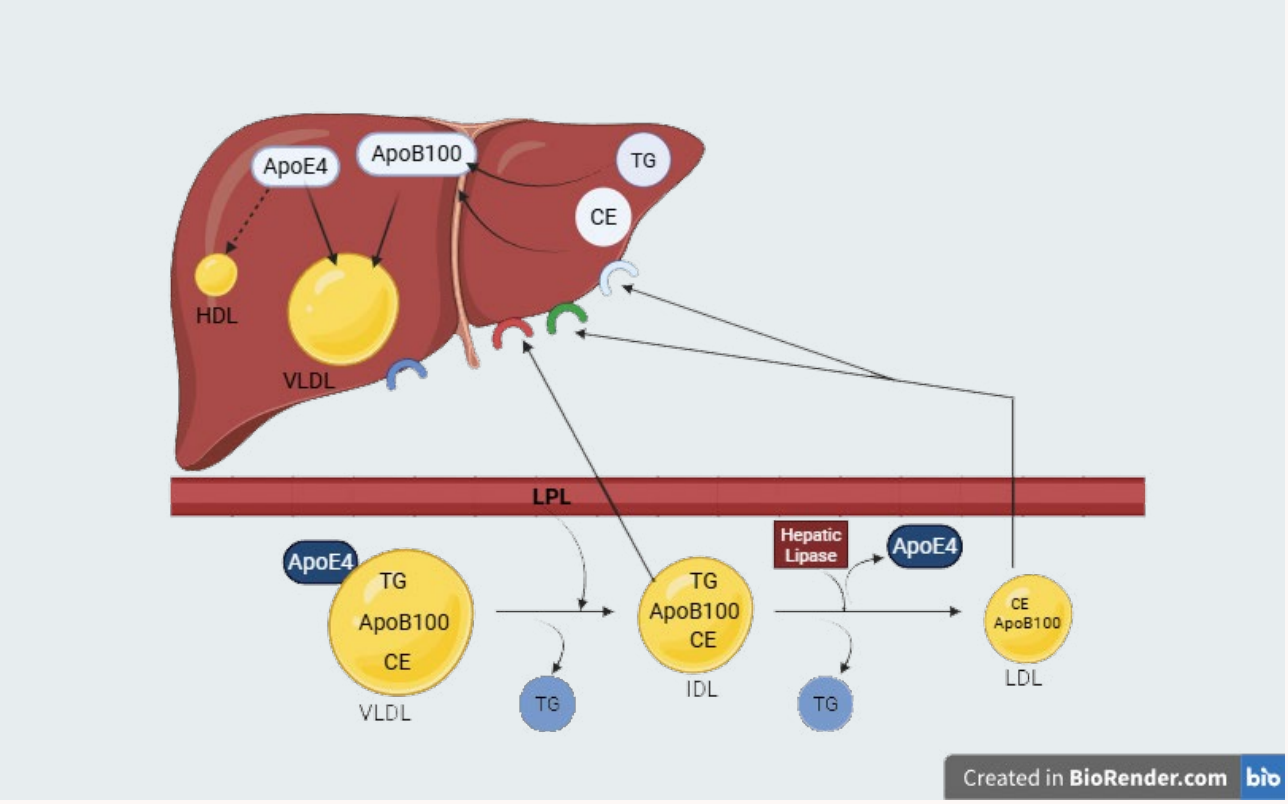

Figure 3. ApoE4 mediated VLDL-Metabolism.

In the liver, cholesterol esters and triglycerides are packaged with ApoB-100, a key structural component of VLDL and a ligand for the LDL-R, thereby facilitating the clearance of circulating lipoproteins. Newly synthesized ApoE4 exhibits a preferential binding affinity for VLDL particles. These VLDL particles are secreted into circulation and deliver triglycerides to peripheral tissues, a process mediated by lipoprotein lipase (LPL)-induced hydrolysis. This enzymatic removal of triglycerides leads to the conversion of VLDL into IDL. ApoE present on IDL enables recognition and uptake by receptors such as LDL-R and LRP1, promoting their clearance from the bloodstream. However, a subset of IDL particles undergoes further triglyceride hydrolysis, facilitated by hepatic lipase, leading to the formation of LDL (Feingold, K. R., 2024). The resulting LDL particles can then interact with various hepatic receptors, including LDL-B, LDL-E, etc. CE- Cholesterol ester, LDL-R- LDL Receptor, LPL- Lipoprotein Lipase, IDL- Intermediate-Density Lipoprotein, LRP-1- LDL Receptor Related Protein 1. (The ApoE4-mediated VLDL metabolism in the above figure emphasizes the preferential binding of ApoE4 to VLDL).

Understanding the Diet: Role in CVD

The role of lipids and lipoproteins in cardiovascular disease (CVD) is well established. Saturated Fatty Acids (SFA) are found in foods like butter, cheese, and red meat. They increase LDL cholesterol (LDL-C), which is a risk factor for CVD. Additionally, they modestly increase HDL cholesterol (HDL-C), which is considered protective, but the overall effect leans towards a negative impact due to the pronounced LDL-C rise (42). Monounsaturated and Polyunsaturated Fatty Acids (MUFA and PUFA) are fats, found in olive oil, nuts, and fatty fish, and are beneficial as they reduce LDL-C levels. PUFAs, particularly omega-3 fatty acids, also have anti-inflammatory properties, making them heart-protective. Replacing SFAs with MUFAs and PUFAs is a key dietary recommendation for lowering CVD risk (43). Trans Fatty Acids (TFA), primarily from partially hydrogenated oils and processed foods, increase LDL-C and decrease HDL-C. This dual negative effect makes TFAs particularly harmful, and their consumption is strongly linked to an increased risk of CVD. Observational studies have consistently shown that higher dietary intake of trans fats (TFA) increases the risk of cardiovascular disease (CVD) (44). A study summarizing the links between fatty acids and coronary disease found that saturated, monounsaturated, and omega-3 and omega-6 polyunsaturated fats showed little to no strong association with coronary risk, while trans fats were linked to a higher risk. The findings suggest limited support for current guidelines promoting high polyunsaturated fat intake and low saturated fat intake for heart health (45).

Similarly, another most discussed dietary factor is cholesterol. Dietary cholesterol, present in egg yolks and shellfish, increases LDL-C levels, though the effect is modest and varies among individuals. Some people, known as hyper-responders, experience a more significant rise in LDL-C. Current recommendations focus on moderating cholesterol intake rather than strict avoidance (5). The Sydney Diet Heart Study, a randomized controlled trial conducted from 1966 to 1973, evaluated the effects of replacing dietary saturated fats with polyunsaturated fats in men aged 30 to 59 years with a history of coronary artery disease. Participants were randomized into two groups: an intervention group (n=221) that received dietary instructions to substitute animal fats, butter, traditional margarines, and shortenings with safflower oil and safflower oil-based polyunsaturated margarine, and a control group (n=237) that received no specific dietary advice. The dietary intervention aimed to increase polyunsaturated fatty acid (PUFA) intake to approximately 15% of total energy, reduce saturated fat (SFA) intake to below 10%, and limit dietary cholesterol to less than 300 mg per day. Additionally, liquid safflower oil was provided as a dietary supplement. At baseline, serum cholesterol

levels in both groups were approximately 280 mg/dL. After the intervention, cholesterol levels decreased to 267 mg/dL in the control group and to 244 mg/dL in the intervention group. Despite these improvements in lipid profiles, the intervention group demonstrated higher rates of all-cause mortality (17.6% vs. 11.8%; P=0.051), cardiovascular mortality (17.2% vs. 11.0%; P=0.037), and coronary heart disease mortality (16.3% vs. 10.1%; P=0.036) over the five-year follow-up period. The paradoxical increase in mortality observed in the intervention group, despite favorable changes in cholesterol levels, raises important concerns. One possible explanation involves the use of safflower oil-based margarine, which may have contained trans fatty acids – a byproduct of partial hydrogenation processes common at the time. Trans fats have since been recognized as detrimental to cardiovascular health, potentially offsetting the anticipated benefits of reduced saturated fat intake (46). These findings underscore the complexity of dietary fat modification strategies and highlight the need for careful consideration of the type and source of fats used in dietary interventions.

Carbohydrates (CHO) are the simple sugars and low-quality carbohydrates that contribute to an increase in triglycerides (TGs). High triglyceride levels are associated with a greater risk of metabolic disorders. Complex carbohydrates like whole grains and legumes are preferred as they have a neutral or beneficial impact on lipids. Trials replacing saturated fat with mainly carbohydrates did not lower CVD risk. However, these carbohydrates were often unspecified and included sugars and refined carbs. Observational studies show that whole-grain carbohydrates can reduce CVD risk when they replace saturated fat (47,48). Dietary fiber consists of non-digestible carbohydrates such as non-starch polysaccharides, cellulose, pectins, hydrocolloids, fructo-oligosaccharides, and lignin. Soluble forms of fiber are found in fruits and vegetables, and insoluble fiber is found in cereals and whole-grain products and helps lower LDL-C by reducing intestinal cholesterol absorption, leading to improved lipid profiles and a reduced risk of CVD. Studies discussed that it reduces cholesterol absorption in the small intestine, which lowers cholesterol in chylomicrons and reduces the amount delivered to the liver. This decrease in liver cholesterol boosts LDL receptors, leading to lower LDL-C levels in the blood (49).

Similarly, phytosterols are naturally present in plant-based foods. Plant sterols and stanols (phytosterols/phytosterols) are known to lower LDL cholesterol levels. Foods with these compounds are commonly used to reduce cholesterol and the risk of atherosclerosis (50). Studies suggest that phytosterols and phytosterols regulate cholesterol metabolism through genes like ABCA1, ABCG5/G8, SREBP-2, and LXR. These genes are involved in LDL cholesterol regulation, which is linked to cardiac diseases. Despite the absence of ABC transporters, phytosterols can still inhibit cholesterol absorption, affecting processes like cholesterol esterification, lipoprotein assembly, and synthesis (50). Phytosterols raise safety concerns beyond cardiovascular effects. Studies have shown that phytosterol-rich vegetable oil can make red blood cells less flexible, shortening rat lifespan, and leading to a ban on such foods in Canada in 2003 (51). In vivo studies also suggest phytosterols may negatively affect hormonal and reproductive systems in rats and goldfish, though this has not been proven in humans. To investigate the effects of lipid-soluble (pro) vitamins, a study on 185 healthy volunteers was conducted, but found no impact on reproductive hormones after a year of phytosterol consumption (52). Additionally, phytosterols may interfere with the absorption of fat-soluble vitamins like vitamin E and β-carotene, prompting recommendations to use natural sources of β-carotene (53). Table 5 outlines the effects of various dietary constituents on lipid and lipoprotein levels, highlighting their roles in cardiovascular health.

Table 5. Summary of the effect of dietary constituents on Lipid and Lipoproteins (5)(Feingold, 2021).

Sl. No.	Diet constituents	Effect on the Lipid and Lipoproteins
1	SFA	Increase LDL-C and modest increase HDL-C
2	MUFA and PUFA	Decrease LDL-C
3	TFA	Increase LDL-C and decrease HDL-C
4	Cholesterol	Increase LDL-C
5	Carbohydrates	Increase TGs particularly simple sugars
6	Fiber	Decrease LDL-C
7	Phytosterols	Decrease LDL-C
SFA = saturated fatty acids; MUFA = Monounsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids		

Dietary guidelines consistently recommend reducing saturated fat (SFA) intake due to its association with cardiovascular disease (CVD) in observational studies. However, meta-analyses show mixed results, suggesting the impact depends on what replaces SFA in the diet. Replacing SFA with polyunsaturated fats (PUFA) reduces CVD risk, while replacement with carbohydrates generally shows no benefit unless they are high-quality carbohydrates. Additionally, the source of SFA matters, with SFA from meat linked to increased CVD risk, but SFA from dairy shows a protective effect (54,55).

Pharmacological Interventions

In addition to dietary strategies, various pharmacological agents are employed to manage lipid levels in individuals with elevated CVD risk, including those with the APOE4 genotype. Statins (e.g., atorvastatin, simvastatin) inhibit HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis, leading to reduced LDL-C levels (56). There are cholesterol management guidelines that recommend using two additional medicines besides statins to further lower LDL cholesterol and reduce the risk of heart disease. One is ezetimibe, which blocks cholesterol absorption and helps reduce LDL levels when used with statins, without major safety concerns. It is also helpful for people who have side effects from statins, like muscle pain, as it can be combined with a lower statin dose. The other option includes proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors like alirocumab and evolocumab, which are approved to lower LDL cholesterol effectively (57). Ezetimibe inhibits intestinal cholesterol absorption by targeting the Niemann-Pick C1-Like 1 (NPC1L1) protein. PCSK9 inhibitors (e.g., evolocumab, alirocumab) enhance LDL receptor recycling, significantly lowering plasma LDL-C. Omega-3 fatty acid formulations (e.g., icosapent ethyl) are also used to modulate triglyceride levels. These agents may have varying efficacy in ApoE4 carriers due to altered lipoprotein processing, underscoring the importance of integrating pharmacogenetic data into treatment plans (58,59). Studies on pharmacological interventions are still evolving, and more research is needed to fully understand the underlying mechanisms. However, the pharmacological interventions, through targeted drugs and their specific mechanisms of action, effectively modulate key biological processes to treat various conditions, offering tailored therapeutic benefits.

Linking APOE Genotype and Diet in Cardiovascular Health

Diet fatty acids and cholesterol are major targets for reducing plasma total and LDL cholesterol as a strategy to decrease cardiovascular disease risk. Individuals with the APOE4 allele show a more significant reduction in LDL-C levels in response to lower dietary fat and cholesterol intake compared to those with the APOE3 or APOE2 alleles (60). Similarly, genetic polymorphisms in other genes can influence how lipids and lipoproteins respond to dietary changes. Clinical conditions also play a role; for instance, the lipid-lowering effects of a low-cholesterol, low-SFA diet are less pronounced in obese individuals (61). Therefore, dietary effects vary among individuals, and clinicians must monitor each patient's response to tailor dietary recommendations.

Research suggests that tailoring diets based on an individual's APOE genotype can significantly improve health outcomes. For APOE2 carriers, a heart-healthy diet with healthy fats, lean proteins, and plenty of fruits and vegetables, along with omega-3-rich foods like fatty fish and walnuts, may offer cardiovascular benefits. APOE3 carriers, with a neutral risk profile, can optimize health through a balanced, nutrient-dense diet that includes lean proteins, whole grains, and colourful fruits and vegetables, while avoiding processed foods. APOE4 carriers, at higher risk for cognitive decline, may benefit from a brain-boosting diet with antioxidant-rich foods and omega-3s to protect against neurodegeneration (34).

Nutrigenomics, the study of how nutrition interacts with genetics, plays a key role in personalized diets. This field explores the complex relationship between genetics and nutrition, highlighting how specific APOE genotypes can influence cardiovascular health and how tailored diets can optimize heart health outcomes. Importantly, gene–environment interactions encompassing dietary patterns, physical activity, and lifestyle choices play a crucial mediating role in determining individual susceptibility and metabolic outcomes in APOE4 carriers. To create a genotype-specific diet plan, routine genetic testing for APOE allele status is required as an initial diagnostic step. Following APOE status identification, specific blood biomarkers including total cholesterol, LDL-C, HDL-C, triglycerides, apolipoprotein B (ApoB), high-sensitivity C-reactive protein (hs-CRP), and markers of oxidative stress should be systematically monitored over time to evaluate treatment efficacy, track longitudinal metabolic trajectories, and dynamically adjust personalized dietary and pharmacological interventions.

In addition to the genetic factors influencing cholesterol metabolism, lifestyle factors such as obesity play a significant role in how the body processes LDL cholesterol. Obesity, particularly abdominal fat, can disrupt hormonal balance and alter gut microbiota, leading to impaired cholesterol absorption and processing. By reducing abdominal fat, individuals can improve insulin sensitivity, reduce inflammation, and enhance the body's ability to process cholesterol more effectively. This not only helps manage cholesterol levels but also makes dietary interventions, such as those tailored to specific APOE genotypes, more impactful. Therefore, combining weight management with personalized dietary strategies is essential for optimizing cholesterol regulation and overall heart health.

Clinical Relevance and Limitations of Evidence

Although numerous observational and mechanistic studies suggest that APOE4 carriers may respond differently to specific dietary interventions, particularly reductions in saturated fat intake, robust clinical trial evidence supporting genotype-based dietary recommendations remains limited. Most existing studies are either cross-sectional, of short duration, or lack genotype stratification with sufficient power to detect meaningful gene-diet interactions.

Furthermore, while this review highlights promising avenues for personalized nutrition, the current evidence base is insufficient to support formal clinical guidelines targeting APOE4 individuals. The Sydney Diet Heart Study, for instance, found paradoxical increases in cardiovascular mortality despite lipid improvements, underscoring that biomarker modulation does not always translate to improved clinical outcomes. These inconsistencies reflect the complex interplay between diet, genotype, and comorbidities, factors often inadequately controlled in many studies.

Therefore, until large-scale, genotype-stratified randomized controlled trials (RCTs) are conducted, recommendations for APOE4-specific dietary modifications should be viewed as hypothesis-generating rather than practice-defining. This limitation must be acknowledged when interpreting the translational significance of current findings.

CONCLUSIONS

The APOE4 allele is a major genetic determinant of cardiovascular disease, being associated with elevated LDL-C levels, increased oxidative stress, and a higher risk of coronary artery disease and stroke. Current evidence demonstrates a clinically significant interaction between APOE genotypes, particularly APOE4, and dietary factors that influences cardiovascular risk. Consequently, personalized nutritional interventions tailored to the APOE4 genotype represent a promising approach for improving lipid profiles and reducing cardiovascular risk. However, further research is needed to better understand gene–nutrient interactions and to strengthen the evidence supporting the routine clinical implementation of genotype-based dietary recommendations. Integrating personalized nutrition into clinical practice may improve preventive and therapeutic strategies for individuals with increased genetic susceptibility to cardiovascular disease.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL APPROVAL

This manuscript is a systematic literature review based on the analysis of fully anonymized, publicly available, peer-reviewed data and secondary research sources. Because no animal or human subjects were directly involved, animal clinical case guidelines do not apply, and institutional ethical approval and written participant consent were not required.

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MOLECULAR AND PHYLOGENETIC CHARACTERIZATION OF THE HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 VIRUS DETECTED IN THE REPUBLIC OF MOLDOVA

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ABSTRACT

Introduction The H5N1 avian influenza virus is one of the most virulent antigenic combinations, including those with zoonotic pathogenic potential. As the number of avian influenza outbreaks has increased globally, a corresponding rise in outbreaks caused by this virus has also been recorded in the Republic of Moldova.

Materials and methods The descriptive study employed epidemiological, clinical and molecular biology (RT-PCR) research methods. The research material consisted of carcasses of domestic and wild birds, as well as blood serum samples collected from various regions of the republic. Identification of the influenza virus in the research material was carried out at the National Reference Laboratory Public Institution National Center for Animal, Plant Health and Food Safety (P.I. NCAPHFS) and the EU Reference Laboratory (EURL, IZSVe). The following diagnostic kits were used for virus identification: the "IndiSpin® Pathogen Kit" (QIAGEN® GmbH for INDICAL BIOSCIENCE) and the "Virotype® Influenza A RT-PCR Kit" (Germany).

Results The molecular structure of HPAI A(H5N1) viruses isolated from the Republic of Moldova demonstrated the presence of genetic mutations in the PB2 protein (E627K and/or D701N) associated with viral adaptation to mammalian hosts and isolates with European H5 viruses belonging to the EA-2021-I, EA-2022-CH, EA-2022-AB, and EA-2023-DB genotypes.

Conclusions The molecular characterization of HPAI A(H5N1) isolated from birds in the Republic of Moldova demonstrated the presence of genetic mutations in the PB2 protein and in the hemagglutinin protein (S133A, S154N, and T156A), which are associated with adaptation to human-type receptors.

Keywords Virus, highly pathogenic, birds, molecular research, avian influenza.

CARACTERISTICA MOLECULARĂ ȘI FILOGENETICĂ A VIRUSULUI GRIPEI AVIARE ÎNALT PATOGEN H5N1 DETECTAT ÎN REPUBLICA MOLDOVA

Introducere Virusul gripei aviare H5N1 reprezintă una dintre cele mai virulente combinații antigenice, inclusiv cu potențial patogenic zoonotic. Odată cu creșterea numărului de focare de gripă aviară la nivel global, s-a înregistrat o creștere a numărului de focare cu acest virus și pe teritoriul Republicii Moldova.

Materiale și metode În prezentul studiu au fost utilizate cercetări epidemiologice, clinice și de biologie moleculară RT-PCR. În calitate de material de cercetare au constituit cadavrele păsărilor domestice și sălbatice și probele de ser sanguin colectate din diferite regiuni ale republicii, iar identificarea virusului gripal din materialul pentru cercetare s-a efectuat în cadrul Laboratorului Național de Referință Instituția Publică Centrul Național Sănătatea Animalelor, Plantelor și Siguranța Alimentară și Laboratorul de Referință UE (EURL, IZSVe). In cercetare au fost folosite chiturile de diagnostic: "IndiSpin® Pathogen Kit" (QIAGEN® GmbH for INDICAL BIOSCIENCE și kitul "Virotype® Influenza A RT-PCR Kit", Germania.

Rezultate Structura moleculară a virusurilor HPAI A(H5N1) izolate din Republica Moldova a evidențiat prezența unor mutații genetice în proteina PB2 (E627K și/sau D701N) asociate cu adaptarea virală la gazde mamifere, precum și a unor izolate înrudite cu virusurile H5 europene aparținând genotipurilor EA-2021-I, EA-2022-CH, EA-2022-AB și EA-2023-DB.

Concluzii Caracterizarea moleculară a virusului HPAI A(H5N1) izolat de la păsări în Republica Moldova a demonstrat prezența unor mutații genetice în proteina PB2 și în proteina hemaglutinină (S133A, S154N și T156A), asociate cu adaptarea la receptorii de tip uman.

Cuvinte-cheie Virus, înalt patogen, păsări, cercetări moleculare, gripa aviară.

INTRODUCTION

The avian influenza virus (AIV) is one of the best-known pathogens, and its persistent circulation has a significant impact on public health, the global economy, the poultry industry, international trade, and the health of wild birds. These are segmented, single-stranded RNA viruses belonging to the *Orthomyxoviridae* family and are highly variable (1). The genetic and antigenic variability of the surface glycoproteins HA and NA is particularly pronounced. To date, sixteen HA (H1–H16) and nine NA (N1–N9) subtypes circulate in wild waterfowl (2), and two new HA subtypes (H17 and H18) and two new NA subtypes (N10 and N11) have recently been identified in bats (3–6).

Compared with the classification based on the antigenic properties of HA and NA, avian influenza viruses are also classified according to their pathogenicity in avian species into two categories: highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI). Most avian influenza viruses are low pathogenic (LPAI) and cause either mild disease or asymptomatic infection, depending on the virus strain and the infected host species. However, certain subtypes, particularly H5 and H7, may evolve into highly pathogenic avian influenza (HPAI) viruses. The current and largest avian influenza epizootic in modern history began with the emergence of the highly pathogenic H5N1 avian influenza virus in Guangdong Province, China, in 1996 (7,8). In 2005, H5N1 viruses belonging to clade 2.2 caused the first major outbreak among wild birds at Qinghai Lake, China. Subsequently, through the migration of wild birds, the virus spread from Asia via Siberia to Europe, the Middle East, and Africa within a few months (9,10). The highly pathogenic avian influenza A(H5N1) virus became widely distributed, affecting both wild birds and domestic poultry across Asia, Europe, and Africa. By the end of 2021, it had also been detected in North America, indicating its further intercontinental spread (11). Outbreaks of highly pathogenic H5N1 avian influenza have caused substantial economic losses to the poultry industry, while sporadic transmission to humans in several countries has highlighted its potential threat to public health. Since 2003, a total of 889 sporadic human cases of avian influenza caused by different clades of HPAI A(H5N1) viruses have been reported across 23 countries (12). Although the exact number of farmed birds affected by these outbreaks is unknown, it is estimated to be in the hundreds of millions (10). Mortality among wild birds due to infection with highly pathogenic avian influenza (HPAI) viruses has historically been considered a rare event. HPAI A(H5N1) infection in wild birds was first reported in Hong Kong in 2002 (2). Wild birds constitute the natural reservoir of influenza A viruses, which typically cause asymptomatic infections.

Based on the above, interest in this disease has also increased in the Republic of Moldova, particularly in late 2023 and early 2024, following the occurrence of mass swan deaths in some bodies of water within the Republic of Moldova. The aim of this descriptive study was to identify and characterize the HPAI H5N1 virus isolated in the Republic of Moldova through whole-genome sequencing and phylogenetic analysis.

MATERIALS AND METHODS

Methodological Approach

A descriptive, quantitative epidemiological study was conducted, combining field surveillance with advanced laboratory diagnostic techniques. Primary diagnostic processing took place at the National Reference Laboratory (P.I. National Center for Animal and Plant Health, and Food Safety, Chisinau),

while confirmatory virological analyses, high-throughput sequencing, and phylogenetic tree reconstructions were conducted in collaboration with the EU Reference Laboratory for Avian Influenza and Newcastle Disease (EURL, IZSVe, Legnaro, Italy). For the laboratory procedures, were followed these steps: Viral RNA Extraction: Performed using the IndiSpin® Pathogen Kit (QIAGEN / INDICAL BIOSCIENCE). Influenza A screening were executed via real-time RT-PCR targeting the Matrix (M) gene using the Virotype® Influenza A RT-PCR Kit (Germany). *Subtype identification* of H5, H7, and N1 genes was carried out using specific primers and probes (Generi Biotech; Primer Design Home of Genesing® H5N1 range), run on a Quant Studio 5 Real-Time PCR System (Thermo Fisher Scientific).

Study Period and Study Area

The study period ran from December 2023 through 2024. Field sampling covered aquatic habitats – such as lakes and river basins – across northern and central districts like Sîngerei, Drochia, Rîșcani, and Florești, alongside poultry populations from backyard holdings. Whenever an H5N1 avian influenza outbreak was triggered a 3 km quarantine zone was established around the area and 59 samples were collected from it to closely monitor the epidemiological situation. Samples from suspected cases were gathered by inspectors from local NFSA (National Food Safety Agency) territorial subdivisions by filling out official minutes. They strictly followed the sampling, packaging, and transport rules laid out in the Regulation on the methodology for sampling, packaging, identification, and transport of samples intended for laboratory examinations in the field of animal health and welfare. All laboratory investigations were carried out at the National Reference Laboratory.

Study Population

The study populations were focused on two main groups: wild aquatic birds living in natural habitats and domestic poultry kept in backyard holdings. Were collected and analyzed a total of 31,820 specimens during the surveillance period. The biological samples gathered included a tracheal and cloacal swabs collected from live birds, as well as parenchymal tissues (including lung, trachea, and brain) retrieved from carcasses and native blood serum used for antibody evaluation. The sampling strategy was divided into two main approaches: passive surveillance- investigations specifically targeting wild bird mortality within natural aquatic habitats and active surveillance – targeted monitoring carried out directly across backyard holdings.

Statistical Analysis

The statistical analysis of data were gathered, organized, and evaluated using descriptive statistical techniques in Microsoft Excel. We determined positivity rates by dividing the number of positive real-time RT-PCR outcomes by the total number of samples tested. Microsoft Excel helped structure our database, compute absolute numbers and percentages, and track epidemiological variables across different districts and sample types. Furthermore, sequence alignments and phylogenetic tree building were carried out using standard bioinformatic tools along with reference sequences supplied by the EURL (IZSVe). Following this, several high-mortality outbreaks were recorded along the Răut River – specifically in Moara de Piatră (Drochia district), Răcăria (Rîșcani district), and Putinești and Lunga (Florești district) – an escalation influenced in part by seasonal weather patterns.

RESULTS

Based on epidemiological investigations, it was determined that in December 2023, cases of mass mortality among mute swans (*Cygnus olor*) were reported in the Republic of Moldova, in the area around the village of Cubolta in the Sîngerei district, from where samples were collected from 21 swans and sent to the P.I. National Center for Animal, Plant Health, and Food Safety (P.I. NCAPHFS). Laboratory results confirmed the presence of the highly pathogenic avian influenza virus (HPAI H5N1). Subsequently, cases of high mortality were recorded along the Răut River (Moara de Piatră village, Drochia District; Răcăria village, Rîșcani District; Putinești village; and Lunga village, Florești District). Subsequently, additional new cases of the disease were recorded in the northern districts of the republic, facilitated by climatic conditions (13). The number of positive avian influenza cases reached 245 out of 31,820 samples submitted to the laboratory from across the entire territory of the Republic of Moldova. The geographic distribution of confirmed cases of avian influenza is shown in Figure 1.

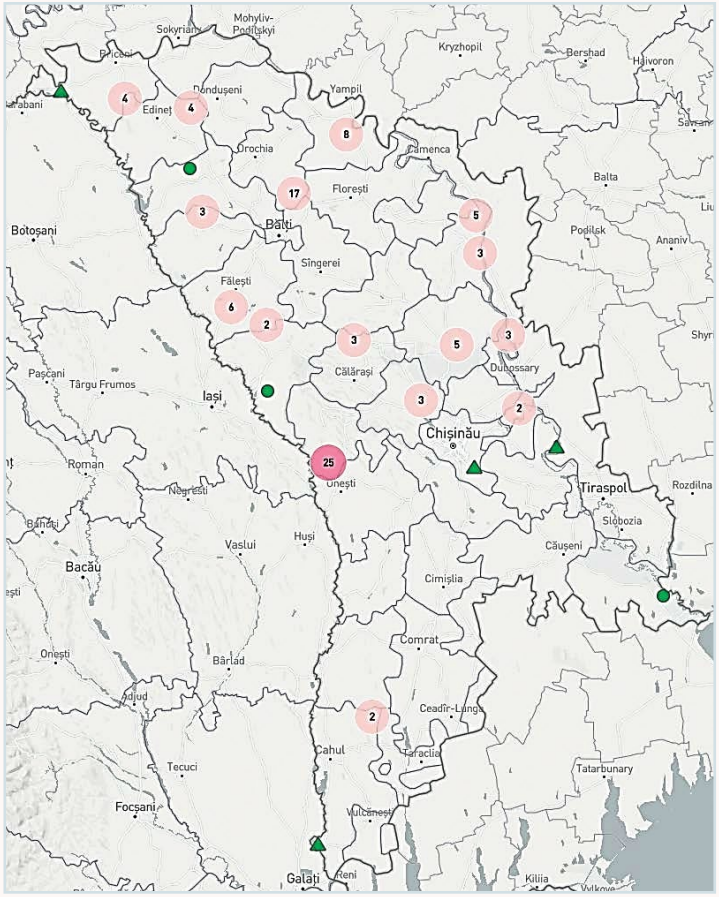

Figure. 1. Geographic distribution of avian influenza outbreaks in the Republic of Moldova during 2023–2024.

An analysis of the geographic locations of avian influenza cases reveals that most cases were recorded in the northern and central districts of the Republic of Moldova. In the southern region, only one case of avian influenza was recorded in the Cahul district; in the western districts, two cases were recorded, specifically in the Căușeni and Ștefan Vodă districts.

The laboratory investigations were aimed at detecting and typing the avian influenza virus, as well as detecting specific antibodies. The Animal Health and Veterinary Medicines Laboratory (LDSA) within the NCAPHFS Public Institu-

tion processed 31,820 samples between December 2023 and November 2024, conducting 57,502 laboratory tests, including blood serum samples using diagnostic methods recommended by the European Union Reference Laboratory for Avian Influenza and Newcastle Disease (Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro, Italy). The testing included the detection of antibodies against avian influenza viruses using the hemagglutination inhibition assay (HI), with the use of reference reagents: inactivated antigen, international reference strain: H5N8-A/turkey/Italy/7898/2014; H5N1-A/mallard/Italy/3401/05; H5N3-A/duck/Italy/775/04; H7N7A/macaw/England/626/80; H7N1-A/turkey/Italy/1067/99; – International reference positive serum – H5N1, H5N8, H7N1, H7N7;- International reference negative serum.

As a result of laboratory testing, the presence of the avian influenza virus was confirmed using the Real-Time RT-PCR method to identify the viral genome (matrix) in all samples submitted. Following the confirmation of avian influenza outbreaks in the Republic of Moldova, the National Agency for Food Safety (ANSA) implemented sanitary and veterinary measures to identify, localize, and eliminate the outbreaks, and during the quarantine periods, a varying number of biological samples (blood serum) were systematically collected and submitted, which included poultry from localities within the protection zones and surveillance zones.

Simultaneously, avian influenza virus identification was performed on 19 samples of pathological material (trachea and brain) at the P.I. NCAPHFS National Reference Laboratory, which confirmed the presence of the highly pathogenic H5N1 subtype in samples collected from wildlife. The results of this descriptive study are presented in Table 1.

Table 1. Identification and Pathogenicity of the Virus.

Nr.	Analysis (Method)	Sub-analysis	Results
19 samples of pathological material (trachea and lungs) collected from swans			
1	Influenza A virus (Reverse Transcriptase Real-Time PCR qualitative / PDP VIR 018 2022 Rev. 7)		Positive
2	Influenza A virus subtype H5 (Reverse Transcriptase Real-Time PCR qualitative / PDP VIR 143 2022 Rev. 3)		Positive
3	Pathogenicity assessment and phylogenetic typing of the Eurasian H5 avian influenza virus by Reverse Transcriptase Real-Time PCR qualitative	H5 Clade 2.3.4.4b	Positive
		H5 CS-HPAI	Positive
4	Influenza A virus – neuraminidase subtype (qualitative PCR Real-Time)	Avian influenza virus – neuraminidase subtype N1	Positive

An analysis of the phylogenetic results presented in Table 1 shows that the examination of the nine genetic segments demonstrates that the HPAI H5N1 viruses from clade 2.3.4.4b identified in the Republic of Moldova belong to two distinct genotypes. Two viruses belong to genotype DA, which began spreading in Europe in September 2023 and has been reported in Austria, Bulgaria, France, Germany, Italy, Romania, and Slovenia. At the same time, seven viruses belong to genotype DI, a new genotype detected in Poland between December 2023 and January 2024, indicating an event of genetic diversification and geographic spread that requires continuous monitoring. These results are presented in Table 2, which shows that in the confirmed avian influenza outbreaks in the Republic of Moldova, the avian influenza virus with the H5N1 antigenic combination was confirmed, with variable characteristics of the viral genome (Table 2) and a predominance of the DA and DI genotypes.

Table 2. Genomic Characterization of the HPAI H5N1 Virus by the EU Reference Laboratory.

Virus	Subtype	Date of Collection	Genotype
A/swan/Moldova/8917-4 24VIR442-1/2023	H5N1	15/12/2023	DI
A/swan/Moldova/8921-2 24VIR442-5/2023	H5N1	16/12/2023	DI
A/swan/Moldova/8956 24VIR442-7/2023	H5N1	18/12/2023	DA
A/swan/Moldova/9020 24VIR442-10/2023	H5N1	20/12/2023	DI
A/swan/Moldova/9045 24VIR442-11/2023	H5N1	21/12/2023	DA
A/swan/Moldova/9046 24VIR442-12/2023	H5N1	20/12/2023	DI
A/swan/Moldova/9048 24VIR442-14/2023	H5N1	20/12/2023	DI
A/swan/Moldova/9115-1 24VIR442-15/2023	H5N1	28/12/2023	DI
A/swan/Moldova/9201-3 24VIR442-19/2023	H5N1	27/12/2023	DI

These results confirm that the molecular structure of viruses isolated in the Republic of Moldova shows that some virus isolates contain molecular markers with zoonotic potential, identified in viral proteins. These findings are summarized in Table 3.

Table 3. Molecular Markers Identified in the Viruses Analyzed by the EU Reference Laboratory.

Protein	Mutation	Effect	Samples
HA	E251K	Increased virus binding to a2-6	A/swan/Moldova/8956_24VIR442-7/2023_H5N1_2023-12-18
	S107R; T108I	Increased virulence in mice; Increased pH of fusion	All
	S133A	Increased pseudovirus binding to a2-6	All
	S154N	Increased virus binding to a2-6	All
	T156A	Increased virus binding to a2-6; Increased transmission in guinea pigs	All
	V182N	Increased virus binding to a2-6; Decreased virus binding to a2-3	All
M1	I43M	Increased virulence in ducks; Increased virulence in mice	All
	N30D	Increased virulence in mice	All
	T215A	Increased virulence in mice	All
NS	C138F	Increased viral replication in mammalian cells; Decreased interferon response	All
	C138F; NS-1:K55E; NS-1:K66E	Enhanced replication in mammalian cells; Decreased interferon response	All
	I106M	Increased viral replication in mammalian cells; Increased virulence in mice	All
	L103F; NS-1:I106M	Increased virulence in mice	All
	P42S	Increased virulence in mice; Decreased antiviral response in mice	All
PA	I38V	PA inhibitor (PAI) baloxavir susceptibility	A/swan/Moldova/9048_24VIR442-14/2023_H5N1_2023-12-20
	N383D	Increased polymerase activity in mammalian cells;	All
	N409S	Increased polymerase activity in mammalian cells; Increased replication in mammalian cells	All
	S37A	Increased polymerase activity in mammalian cells	All

Protein	Mutation	Effect	Samples
PB1	D3V	Increased polymerase activity in mammalian cells; Increased replication in mammalian cells	All
	D622G	Increased polymerase activity in mice; Increased virulence in mice	All
PB2	I292V	Increased polymerase activity in mammalian cells, Increased virulence in mice; Increased polymerase activity in mammalian cells	Seven out of nine
	K389R	Increased polymerase activity in mammalian cells; Increased replication in mammalian cells	All
	L89V; G309 D	Increased polymerase activity in mammalian cells; Increased virulence in mice	All
	L89V; G309 D; T339K; R4 77G; I495V; K627E; A67 6T	Increased polymerase activity in mammalian cells; Increased virulence in mice	A/swan/Moldova/9045_24VIR44 2-11/2023_H5N1_2023-12-21, A/swan/Moldova/8956_24VIR44 2-7/2023_H5N1_2023-12-18
	V598T	Increased virulence in mice; Increased polymerase activity in mammalian cells; Increased replication in mammalian cells	All

Analyzing the data in Table 3, we observe that HA (hemagglutinin) alters receptor specificity (α2,3-avian/α2,6-human) and protein stability; in particular, the E251K mutation increases binding to the α2,6 (human) receptor, and the virus gains affinity for human respiratory cells, posing an increased zoonotic risk. In turn, the S107R and T108I mutations demonstrate increased virulence in mice and result in greater stability, as well as easier activation within the human respiratory tract. For this reason, the S133A mutation results in binding to the α2,6 receptor, which represents an adaptation to mammals, while the S154N mutation results in binding to the α2,6 receptor, which promotes infection in humans. Although the V182N mutation increases binding to α2,6 and decreases binding to α2,3, this shift is indicative of a mutation associated with increased human tropism. Analyzing the data regarding the PB2 protein (Polymerase Basic 2), we note that it plays a critical role in viral replication and adaptation to mammals, and the I292V mutation is associated with increased virulence in mammals and reflects enhanced adaptation to mammalian hosts. At the same time, the mutations L89V, G309D, T339K, R477G, I495V, K627E, and A676T are associated with increased viral replication and virulence in mammals; in contrast, the K627E mutation is one of the best-known mutations associated with adaptation to humans.

DISCUSSION

The results of the descriptive study confirmed the presence and circulation of the avian influenza virus, which, depending on certain circumstances (contact with various bird species, transmission via various vectors such as domestic and wild animals, and humans), is capable of altering certain genes in the viral genome and acquiring new pathogenic properties for other species of birds and animals, as well as posing a risk to public health. These findings are consistent with those reported by other authors (5, 8, 12). Based on data presented by the European Union Reference Laboratory for Avian Influenza & Newcastle Disease, the Istituto Zooprofilattico Sperimentale delle Venezie in Legnaro, Italy, in collaboration with the National Reference Laboratory P.I. NCAPHFS, we can note that the molecular and phenotypic analysis of the H5N1 HPAI virus identified in the Republic of Moldova reveals two distinct genotypes with increased virulence and pathogenicity. Specific genetic changes

in the HA and PB2 proteins resulting from mutations isolated from wildlife (swans) may pose an increased zoonotic risk to humans, and the data obtained through molecular sequencing allow for a rapid and effective assessment of the avian influenza risk to the agri-food sector.

It is also important to note that PB2 contains the most obvious zoonotic mutations that would allow the virus to replicate efficiently in a human host (15, 16). Nevertheless, the avian influenza virus has the potential to increase the number of incursions, given the high viral loads carried by migratory birds (16). It is also encouraging that, to date, the avian influenza virus does not have the ability to cause sustained human-to-human transmission; however, this phenomenon poses a major risk for the future and requires constant and thorough monitoring.

CONCLUSIONS

The molecular characterization of HPAI A(H5N1) viruses isolated from wild birds and domestic poultry in the Republic of Moldova during 2023–2024 demonstrated the presence of genetic mutations that distinguish these viruses from the genetic profiles of H5N1 viruses identified in several European countries. Genome analysis further revealed mutations in the PB2 protein (E627K and/or D701N) associated with viral adaptation to mammalian hosts, while also confirming the genetic relationship of the Moldovan isolates with European H5 viruses belonging to the EA-2021-I, EA-2022-CH, EA-2022-AB, and EA-2023-DB genotypes.

Analysis of the hemagglutinin (HA) protein identified the receptor-binding mutations S133A, S154N, and T156A, which are associated with adaptation to human-type receptors, in the majority of H5N1 viruses circulating in the Republic of Moldova. These mutations were likewise detected in viruses circulating in several European countries during the same period, highlighting the continued evolution of H5N1 viruses and emphasizing the importance of ongoing molecular surveillance to assess their zoonotic potential.

In this context, continuous surveillance and epidemiological monitoring of avian influenza remain national priorities for both the poultry sector and public health. Effective prevention and control of HPAI depend on robust laboratory diagnostic capacity, accurate molecular characterization, and the timely implementation of veterinary public health measures aimed at mitigating the spread of avian influenza viruses.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

In conducting the research and reporting the results, the authors complied with all requirements of professional ethics.

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SELF-RATED HEALTH IN MOLDOVA: A STUDY BASED ON THE GENERATIONS AND GENDER LIFE-COURSE SURVEY

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ABSTRACT

Introduction Subjective health serves as an indicator of quality of life and is a reliable predictor of mortality trends, particularly in older age groups. Its determinants are influenced by physical and mental well-being and are positively linked to overall health. Subjective health is mainly measured using a representative sample, in which respondents rate their health, usually on a scale from very good to very bad.

Materials and methods This research primarily relies on life-course data from the Generations and Gender Survey, conducted in two waves in 2020 and 2024–2025. Thus, a panel analysis was conducted to assess changes in subjective health across the 5-year cohorts during the period analysed.

Results Subjective health has declined more than improved across all studied generations, with a notably greater reduction among the middle-aged group born between 1965 and 1980, in both males and females. The decline in subjective health among these groups aligns with a rising prevalence of chronic diseases as the interviewees age.

Conclusions The research addresses a gap in the study of subjective health in Moldova by applying panel data analysis, whereas previous research has predominantly used a cross-sectional approach. The article's findings highlight the need for targeted policies to prevent morbidity and reduce mortality, particularly among vulnerable cohorts.

Keywords Subjective health, population health, healthy life expectancy, self-rated health.

SĂNĂTATEA AUTOEVALUATĂ ÎN REPUBLICA MOLDOVA: UN STUDIU BAZAT PE CHESTIONARUL GENERAȚII ȘI GEN DE-A LUNGUL CURSULUI VIEȚII

Introducere Sănătatea subiectivă reprezintă un indicator al calității vieții și un predictor fiabil al tendințelor mortalității, în special la grupele de vârstă înaintată. Determinanții acesteia sunt influențați de starea de bine fizică și mentală și corelează pozitiv cu starea generală de sănătate. Sănătatea subiectivă este măsurată, de regulă, pe baza unor eșantioane reprezentative, în cadrul cărora respondenții își evaluează propria stare de sănătate, de obicei pe o scală de la foarte bună la foarte rea.

Materiale și metode Cercetarea se bazează în principal pe datele privind parcursul vieții obținute în cadrul Anchetei Generații și Gen (Generations and Gender Survey), desfășurată în două valuri, în anii 2020 și 2024–2025. Astfel, a fost realizată o analiză de tip panel pentru a evalua modificările sănătății subiective în cadrul cohortelor de 5 ani pe parcursul perioadei analizate.

Rezultate Sănătatea subiectivă s-a deteriorat într-o măsură mai mare decât s-a îmbunătățit în toate generațiile studiate, cu o reducere semnificativ mai pronunțată în rândul persoanelor de vârstă mijlocie, născute între anii 1965 și 1980, atât la bărbați, cât și la femei. Scăderea nivelului de sănătate subiectivă în aceste grupuri este în concordanță cu creșterea prevalenței bolilor cronice odată cu înaintarea în vârstă a respondenților.

Concluzii Studiul contribuie la acoperirea unui gol în cercetarea sănătății subiective din Republica Moldova prin utilizarea analizei datelor de tip panel, în timp ce cercetările anterioare s-au bazat predominant pe abordări transversale. Rezultatele evidențiază necesitatea elaborării unor politici țintite pentru prevenirea morbidității și reducerea mortalității, în special în rândul cohortelor vulnerabile.

Cuvinte-cheie Sănătate subiectivă, sănătatea populației, speranța de viață sănătoasă, sănătate autoevaluată.

INTRODUCTION

Subjective health is one of the key indicators in studying population health, but also in developing health policies. Since subjective health is a self-rated state of physical and mental well-being, it may not necessarily correspond to a diagnosed or objectively measured state of health. However, given that subjective health is positively associated with overall health (1), serving as an indicator of the future mortality trends (2), its assessment is much more affordable and easier to perform than evaluating the population's objective health. Subjective health is often assessed through interviews within a nationally or locally representative sample. The questions generally concern basic demographic information, self-assessed health (rated from "very bad" to "very good"), and diagnosed conditions, if any.

The literature clearly indicates the connection between low self-rated health and the presence of chronic diseases, functional limitations or disability (3), while education, income, and other socioeconomic factors have distinct influences on self-rated health (4). On the other hand, these characteristics influence health inequalities between genders and different population strata (5, 6). Additionally, the evidence shows a connection between physical and mental well-being, overall quality of life, and self-rated health, especially among the elderly (7).

In the context of Moldova, self-rated health allows for investigating geographical differences in overall well-being since the population is registering a visible discrepancy in health, morbidity, and life expectancy between regions and rural-urban localities (8), as well as by other socioeconomic factors and education, especially among the elderly (9, 10). Of course, this also results from factors such as health system capacity, health policies, or bureaucratic efficiency (11). Additionally, in the context of outmigration in Moldova, which shapes the population's health through the 'healthy migrant effect' (12), migration remittances appear to improve the health of children and the elderly left behind (13, 14).

Moldova has registered a similar pattern of subjective health to that of countries in the region, with self-perceived health visibly worsening, particularly among the working-age population (15). Even though life expectancy in good health and without disabilities has increased at a slightly higher rate than life expectancy at birth in recent decades, the gap between Moldova and Central and Western European countries remains significant (16).

The aim of this study is to examine changes in subjective health across cohorts and over time in Moldova using longitudinal data from the Generations and Gender Survey (GGS). While previous studies on subjective health in Moldova have primarily relied on cross-sectional data, this study emphasises how self-rated health evolves longitudinally across generations. This study hypothesises that subjective health tends to worsen with age, with variations by sex and cohort due to differences in lifetime risk exposure. As the population's health is a key factor shaping cause-of-death mortality trends, studying subjective health may improve policy design to reduce the risk of developing certain conditions and address regional disparities.

MATERIALS AND METHODS

Study Design and Data Sources

The research was based on data from the GGS life-course national representative survey, conducted in two waves: the first wave in 2020 and the second wave in 2024–2025. The study used information from both survey periods to assess changes in population health status over time. Additional demographic data, including population age and sex structure and death counts by age at death, were obtained from the database of the National Bureau of Statistics.

Study Population and Variables

The selected variables included date of birth, sex, self-reported subjective health status, and self-declared health conditions. The first survey wave included 8956 observations, while the second wave included 5559 observations. A total of 4815 respondents participated in both survey waves and were included in the longitudinal analysis.

Healthy Life Expectancy Estimation

Healthy life expectancy was estimated using Sullivan's method by combining period life tables for 2020 and 2024 with the prevalence of the population reporting good health status from the GGS survey. Life tables were constructed using 5-year age groups, with the final open-ended age interval of 85 years and older. Healthy life expectancy at age x was calculated according to the following formula:

$$e_x^{healthy} = \frac{T_x^{healthy}}{l_x};\ T_x^{healthy} = \sum_{y \geq x} L_y^{healthy};\ L_y^{healthy} = L_y \times \pi_x$$

where:

$e_x^{healthy}$ – the healthy life expectancy at age x;

$T_x^{healthy}$ – the sum of the life table person-years above age x;

l_x – life table survivors at age x;

L_y – the life table person-years lived within the age-group interval;

π_x – the prevalence of the population self-identified as healthy.

Estimation of Health Prevalence for Older Age Groups

Because the GGS sample included individuals aged 15–79 years in the first wave, the prevalence of good health status for the older age groups (80–84 and 85+ years) was estimated using a relative extrapolation method, according to the following formula:

$$\pi_{x+1} = \pi_x \times r;$$

where:

$$r = \frac{\pi_x}{\pi_{x-1}}$$

Longitudinal Analysis of Subjective Health Changes

A panel analysis was conducted among respondents who participated in both GGS waves. Incomplete questionnaires were excluded before analysis. Self-assessed health responses were recategorized into three groups: (a) "very good" and "good"; (b) "fair" and "bad"; and (c) "very bad". Differences between responses were classified as improvement or deterioration of subjective health status. Participants were subsequently grouped into 5-year birth cohorts.

RESULTS

Table 1 presents the change in life expectancy and healthy life expectancy for 2020 and 2024 at ages 0, 15, and 65. During this period, life expectancy increased among both males and females at birth of 1.6 and 2.5 years, respectively, driven by high mortality during the COVID-19 pandemic and subsequent improvements. This increase in life expectancy was primarily driven by declines in mortality among the middle-aged and elderly, partially through the 'survivor effect'. At the same time, the healthy life expectancy, calculated for the same population sample at two time points, increased by 1.1 years in females, while it remained unchanged in males. This modest increase in healthy life expectancy is primarily related to the increase in life expectancy.

Table 1. The healthy life expectancy at the 1st and 2nd waves of the GGS Survey.

	2020			2024		
Age	0	15	65	0	15	65
Females						
e_x	73.9	59.7	14.8	76.4	62.5	16.6
$e_x^{healthy}$	67.3	52.3	9.8	68.4	53.4	9.7
Males						
e_x	65.9	51.7	11.3	67.5	53.6	12.9
$e_x^{healthy}$	62.5	47.5	8.2	62.5	47.5	8.7

Source: The National Bureau of Statistics and the GGS

Figure 1 shows a certain degree of stability in individual health perception between the first and second GGS waves. In both males and females, the largest proportion of the population within the overall sample estimated their health as 'good' and 'fair', while the number of people in 'bad' health was visibly lower. At the same time, the number of persons who reported their health as 'very good' or 'very bad' was a smaller percentage.

The results emphasise a downward transition in self-rated health among the interviewees between the GGS waves, with the largest shift from 'good' to 'fair' and from 'fair' to 'bad'. A smaller proportion of the people interviewed reported improved health, more often from 'fair' to 'good' and from 'bad' to 'good, although the number of those who reported a radical improvement or worsening of self-rated health was insignificant between the first and second GGS waves.

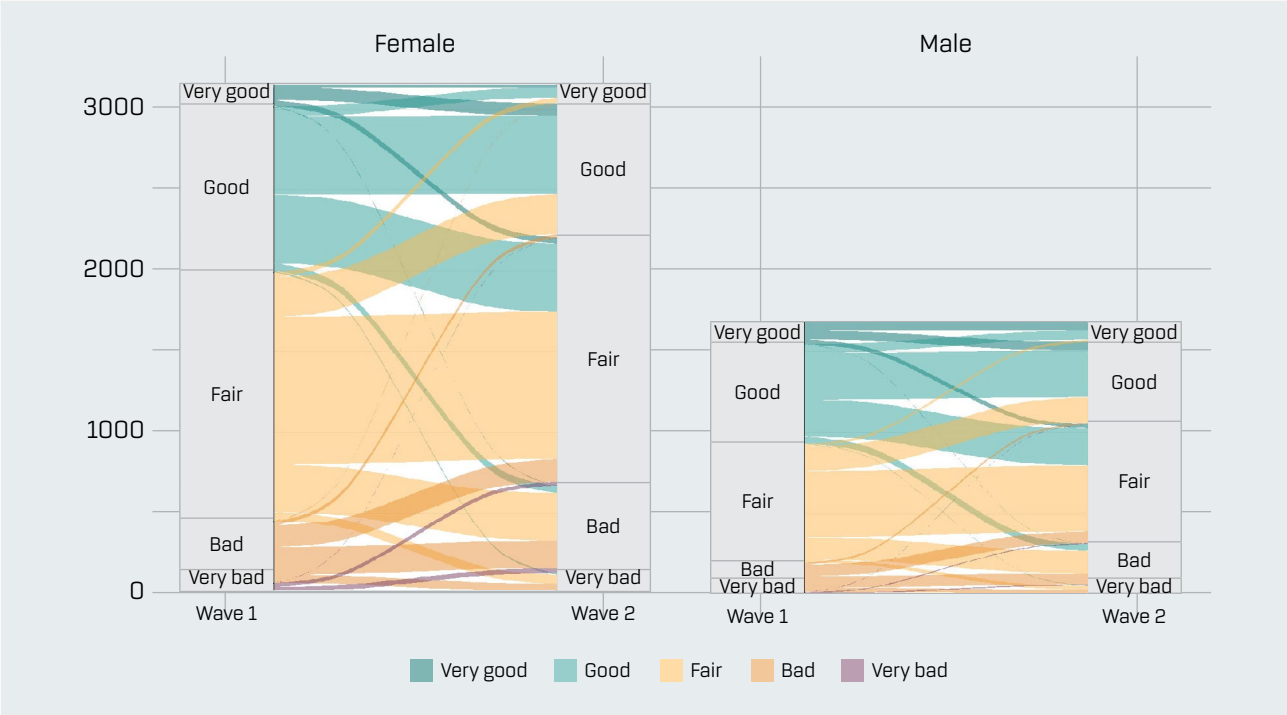


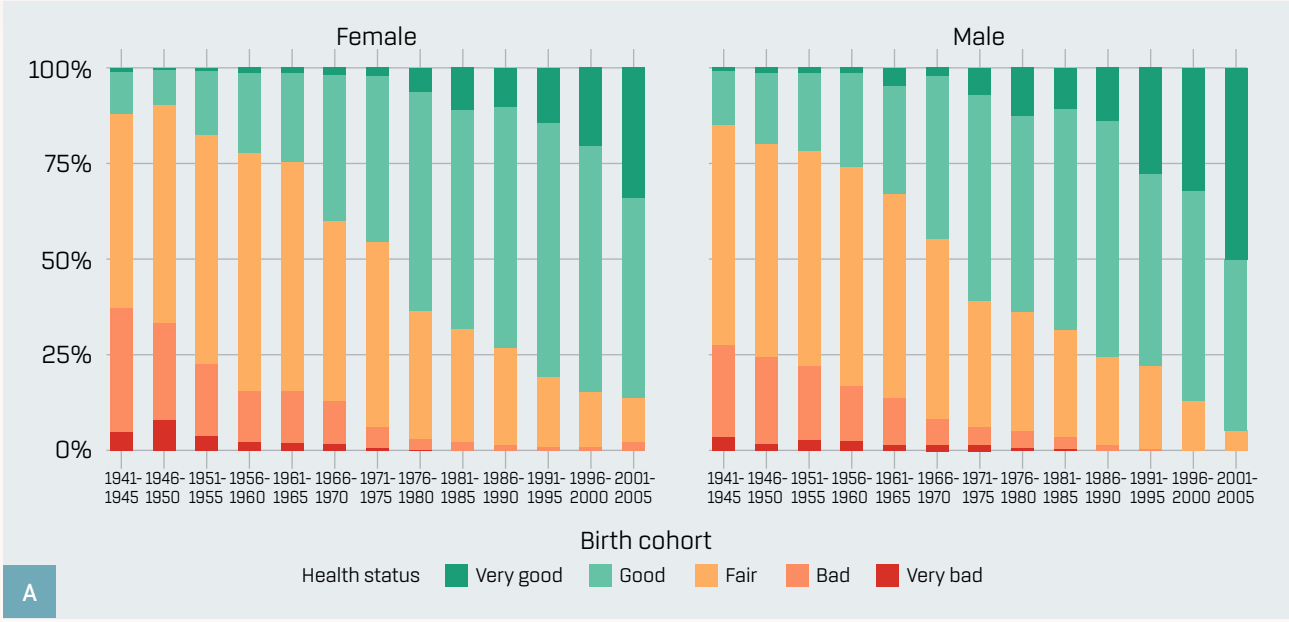
Figure 1. Changes in self-rated health between the first and second waves of the GGS Survey in Moldova.

Source: The GGS Survey

The distribution of self-rated health, from "very good" to "very bad" at both GGS waves (Figure 2) primarily depends on interviewees' year of birth, with younger generations reporting their health as good or very good, and rarely as fair or bad. Conversely, older cohorts tend to report their health as "good" or "fair", or as "bad" and "very bad" among the elderly. The data reveal a noticeable decline in self-rated health during the analysed period, across all generations studied.

Across both waves, a gender gap in subjective health was observed, with men reporting better health than women did at younger ages – a pattern also observed in other research (2). However, since men and women were approximately 4 years apart at the time of the interviews, the gap between the sexes narrowed.

When examining self-rated health across generations, those born before 1965 reported worse health status than younger cohorts, a shift evident when comparing the 1961-1965 and 1966-1970 cohorts. Nevertheless, a similar shift could be observed when contrasting generations born between 1971-1975 and 1976-1980.



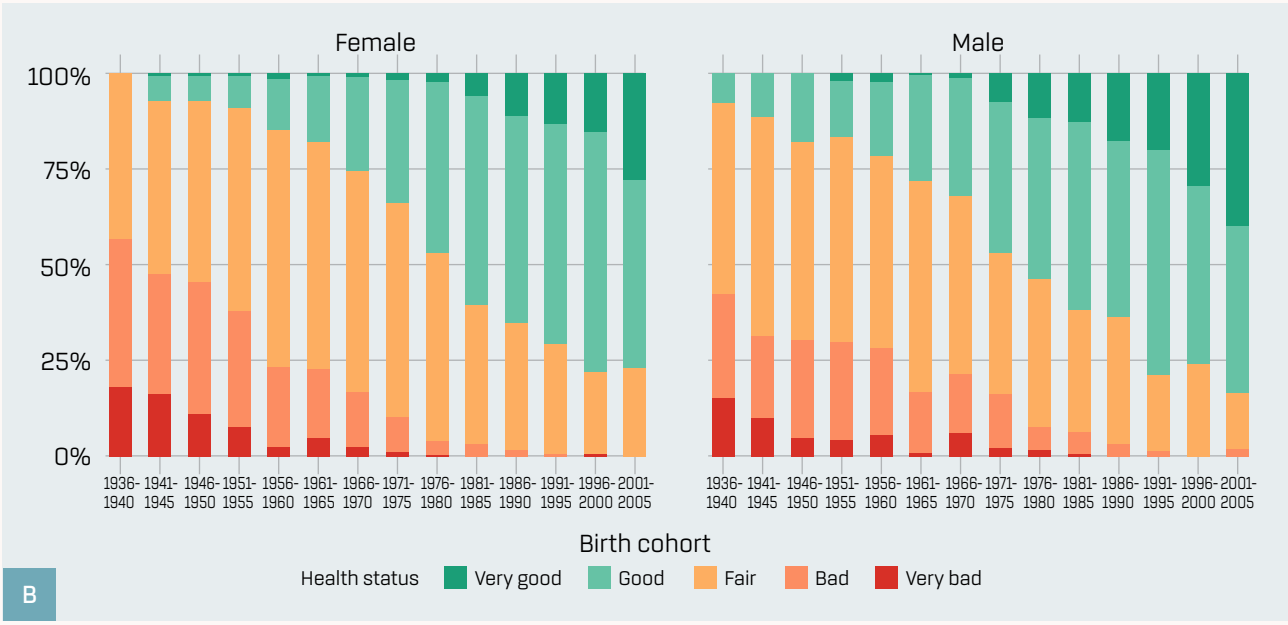


Figure 2. Self-rated health by birth cohort and sex during the first (A) and second (B) waves of the GGS Survey in Moldova.

Source: The GGS Survey

Figure 3 presents the change in subjective health during the second wave of GGS compared to the first one. During this period, the majority of respondents reported their health status as unchanged, regardless of sex. At the same time, a visible proportion of interviewees reported a decline in self-rated health, especially among older generations, while the share reporting improved health was smaller.

The largest increase in subjective health was observed among young females in the 2001-2005 birth cohorts, accompanied by a smaller decline, while a broader decline in self-reported health was observed during the period analysed among generations transitioning to middle age.

Thus, the generations born between 1965 and 1980 show the greatest worsening in subjective health. The elderly cohorts show the lowest level of health improvement but also a lower degree of health deterioration than the generations born between 1966 and 1980, given that in both waves of the study, the 1941–1965 cohorts reported a lower degree of subjective health. Therefore, differences in subjective health by year of birth highlight the effect of age on the population's health status.

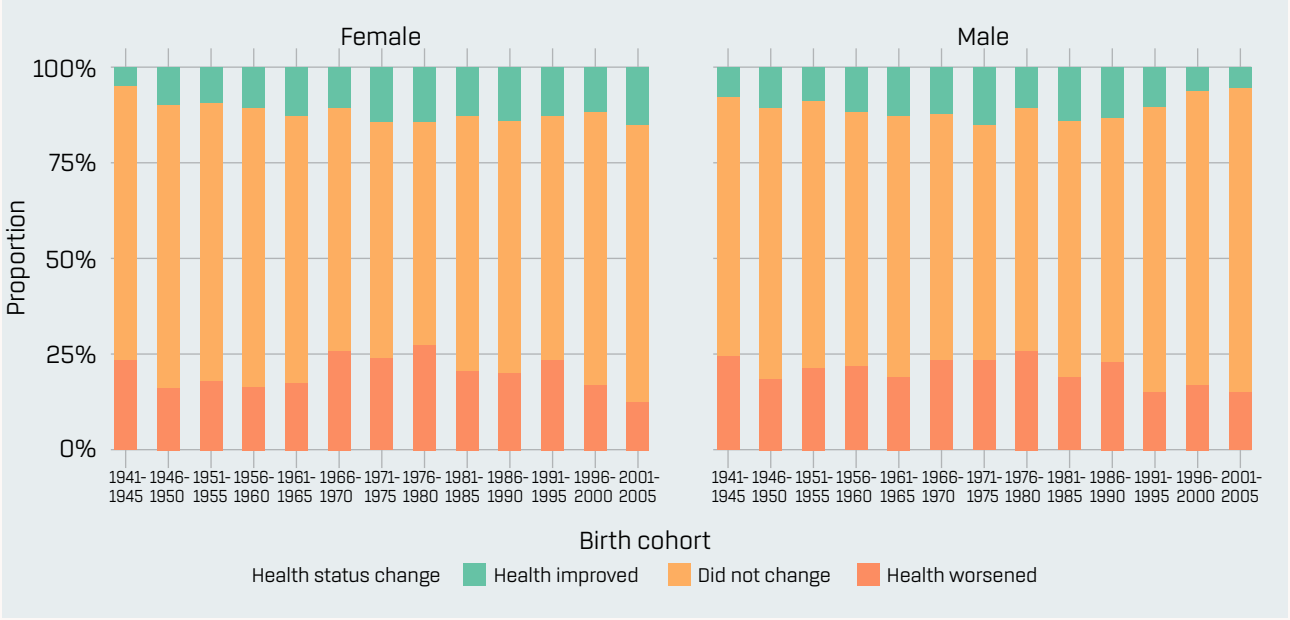


Figure 3. Change in subjective health status by birth cohort and sex between the 1st and 2nd GGS Survey waves, %.

Source: The GGS Survey

Subjective health is primarily associated with overall health and population morbidity. Figure 4 displays changes in population morbidity by age of the people interviewed during the GGS. Among the young population aged 20 years or less, over 80% of interviewees reported no conditions, as their health was predominantly rated as "good" or "very good". However, as the population ages, the proportion of people without disease and in good health decreases sharply. With age, the prevalence of circulatory system diseases and arterial hypertension becomes more prominent, while the prevalence of diabetes, cancer, ulcers, etc., is also notable. Men tend to experience cardiovascular diseases, diabetes, and hypertension earlier than women, who develop hypertension more gradually but show high prevalence at older ages.

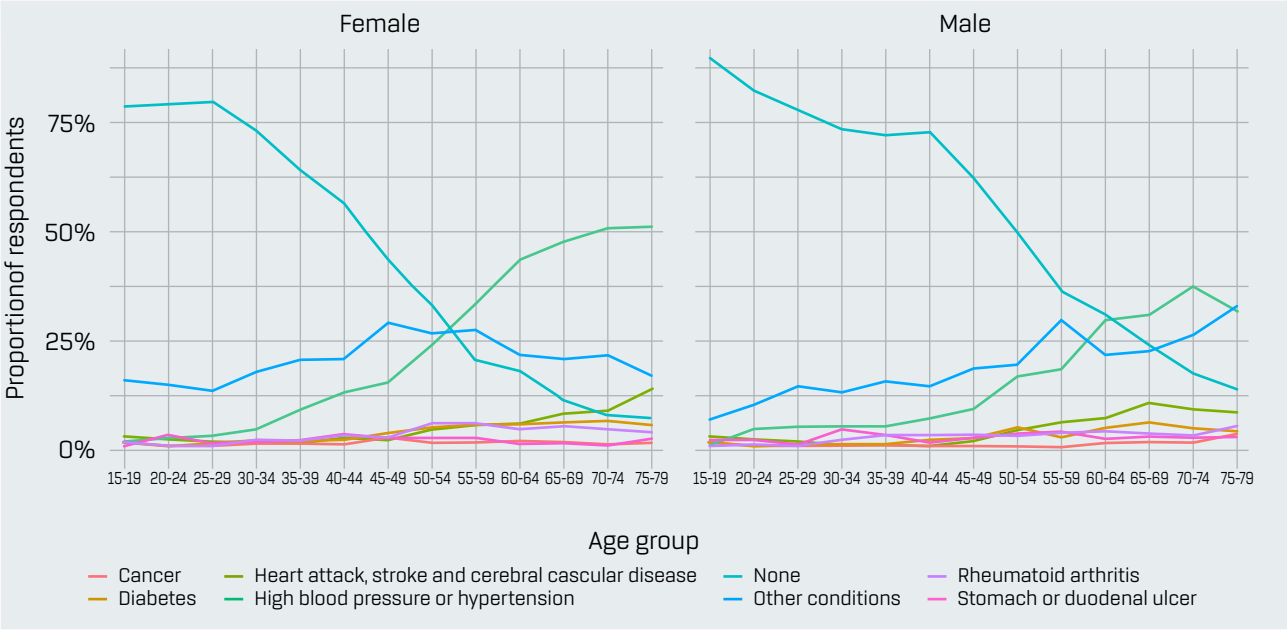


Figure 4. The prevalence of health conditions at the 1st wave of the GGS Survey, by age and sex

Source: The GGS Survey.

Note: Other conditions include asthma, cataracts, chronic lung diseases such as chronic bronchitis or emphysema, fractures, high blood cholesterol, Parkinson's disease, osteoarthritis, and other rheumatic conditions, and other conditions not declared.

The results highlight that chronic diseases are concentrated mainly in older age groups, even though some conditions, such as hypertension, are increasing sharply since the middle ages. However, as younger generations age and become less exposed to the risks that lead to chronic disease, the trend toward improving population health and reducing mortality is likely to continue.

DISCUSSION

Even though population health is challenging to investigate, since health is dynamic throughout life (20), self-rated health provides a broader view on how health and the prevalence of certain conditions evolve among individuals over time and with age. This article primarily examines changes in subjective health among the interviewees between the first and second waves of the GGS Survey, since it offers longitudinal data.

The results of the study emphasise worsening self-reported health with advancing age, with a sharper decline observed in the middle-aged population in both sexes. Particularly, the most affected population is born between 1965 and 1980, among whom an increase in the prevalence of hypertension and other circulatory system diseases is observed. Men tend to experience an earlier onset of cardiovascular diseases, diabetes, and hypertension compared to women, who develop hypertension at a slower pace but have a high prevalence in older age groups.

The observed deterioration in self-rated health among the 1965–1980 generation may be due to the age-specific progression of chronic conditions, often associated with lifestyle and exposure to risk factors over the lifetime (21). Beyond biological health, the middle-aged population may experience increased stress due to intensified role strain, which ultimately reflects on self-perceived

health. Thus, the observed decline in self-rated health might represent a combination of objective health deterioration associated with biological ageing and escalating social demands (22).

Between the two waves of the GGS Survey (2020 and 2024-2025), life expectancy increased significantly, primarily due to high mortality during the COVID-19 pandemic and its subsequent decline in the following years. The main contribution to the increase in age components of life expectancy was observed in the middle-aged and elderly populations. At the same time, the healthy life expectancy, in which people live in good health and without disabilities and chronic or degenerative diseases, has improved only in females, while in males it has remained unchanged. This might be explained through the possible 'survivor effect', especially among the elderly population, since this indicator was calculated for the same population sample.

The findings of this research align with previous studies on Eastern Europe, where differences in self-rated health by sex and across cohorts evolve unevenly, reflecting the influence of historical and socioeconomic contexts (6). Post-Soviet countries emphasise the impact of socioeconomic disruptions, as well as alcohol and tobacco consumption, on population health and mortality during the 1990s, particularly among men (23, 24). From a life-course perspective, exposure to prolonged socioeconomic adversity during critical stages of adulthood may have had lasting consequences for health, contributing to the elevated prevalence of chronic conditions observed in these cohorts (25). Further, as younger generations in these countries have greater access to higher education and are less likely to be engaged in manual occupations, widening intergenerational inequalities in self-rated health may emerge.

Previous research on Moldova has reported a gradual increase in healthy life expectancy (9, 16), largely driven by cohort replacement, as younger generations reach older ages in relatively better health. However, the findings of this study indicate an overall deterioration in self-rated health at the population level, reflecting population ageing. At the same time, younger cohorts report better subjective health compared to older generations. These results highlight the importance of addressing the needs of more vulnerable, older cohorts, while also sustaining the favourable health conditions observed among younger generations.

The presented research has several important limitations that could affect the quality of the results. Firstly, the survey did not include the localities on the left bank of the Nistru River. Secondly, since the first wave of the GGS Survey was conducted during the COVID-19 pandemic public health emergency, with a break during the lockdown, individuals interviewed during this period might have an altered perception of their subjective health (26). Thirdly, a limitation of the GGS dataset for calculating healthy life expectancy is its age range of 15-79, which excludes individuals aged 80 and older. And lastly, the absence of information regarding the reasons for non-participation in the second wave limits the possibility of analysing the subjective health of individuals who passed away between the 1st and 2nd waves.

CONCLUSIONS

The results of the study emphasise a higher decline in subjective health among the middle-aged population born between 1965 and 1980 in both males and females. However, subjective health has registered a greater decline than improvement across all generations studied. The increase in morbidity is also associated with advancement in age, registering a sharp growth since the age of 35, predominantly in circulatory system diseases (heart attack, hypertension, stroke and cerebral vascular diseases), cancer, diabetes, ulcer, and other unspecified conditions.

The GGS Survey provides a unique opportunity to observe changes in subjective health across generations, offering a longitudinal perspective on population well-being. Therefore, the research enhances the existing evidence on subjective health in Moldova by employing panel data analysis, whereas earlier studies have mainly relied on cross-sectional data from the Household Budget Survey. By using a longitudinal approach, we gain a clear understanding of how subjective health develops among individuals over time and with age. Ultimately, the results offer valuable insights for future population health policies aimed at preventing the development of certain chronic diseases and reducing morbidity and mortality, with a focus on specific cohorts. The future research will examine regional differences in subjective health.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS APPROVAL

This study did not require ethical approval, as the research did not involve human participants, animals, or interventions subject to ethical review by a committee.

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DIAGNOSTIC VALUE OF HEMATOLOGICAL INFLAMMATORY MARKERS IN INTRAHEPATIC CHOLESTASIS OF PREGNANCY

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ABSTRACT

Introduction Intrahepatic cholestasis of pregnancy (ICP) is a liver disorder associated with elevated bile acid levels, systemic inflammation, and increased maternal and fetal risks. Identifying accessible and cost-effective inflammatory biomarkers may improve clinical assessment of patients with ICP.

Materials and methods The case-control study included 142 pregnant women: 71 diagnosed with ICP and 71 healthy controls. Hematological inflammatory markers were evaluated, including neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), mean platelet volume (MPV), red cell distribution width (RDW), and emerging ratios such as platelet-to-monocyte ratio (PMR) and C-reactive protein-to-lymphocyte ratio (CLR). Diagnostic performance was assessed using receiver operating characteristic (ROC) curve analysis.

Results NLR and PLR were significantly higher in the ICP group than in the control group ($p<0.0001$). ROC analysis demonstrated moderate discriminatory ability for NLR (AUC=0.707) and PLR (AUC=0.709). Other markers showed limited diagnostic value.

Conclusions NLR and PLR provide additional information regarding systemic inflammation in patients with ICP and may serve as complementary tools in clinical evaluation. Further studies are required to confirm their prognostic and diagnostic relevance.

Keywords Intrahepatic cholestasis of pregnancy, obstetric cholestasis, ICP, hematological inflammatory markers.

VALOAREA DIAGNOSTICĂ A MARKERILOR HEMATOLOGICI DE INFLAMAȚIE ÎN COLESTAZA INTRAHEPATICĂ DE SARCINĂ

Introducere Colestaza intrahepatică de sarcină (CIS) este o afecțiune hepatică asociată cu creșterea concentrației acizilor biliari, inflamație sistemică și riscuri materne și fetale crescute. Identificarea unor biomarkeri inflamatori accesibili și rentabili ar putea îmbunătăți evaluarea clinică a pacientelor cu CIS.

Materiale și metode Studiul de tip caz-control a inclus 142 de femei gravide: 71 diagnosticate cu CIS și 71 gravide sănătoase în lotul de control. Au fost evaluați markerii hematologici ai inflamației, inclusiv raportul neutrofile/limfocite (NLR), raportul trombocite/limfocite (PLR), volumul trombocitar mediu (MPV), amplitudinea distribuției eritrocitare (RDW), precum și indicatori emergenți, cum ar fi raportul trombocite/monocite (PMR) și raportul proteina C reactivă/limfocite (CLR). Performanța diagnostică a markerilor a fost evaluată prin analiza curbelor caracteristicii de operare a receptorului (ROC).

Rezultate Valorile NLR și PLR au fost semnificativ mai mari în grupul cu CIS comparativ cu lotul de control ($p<0,0001$). Analiza ROC a demonstrat o capacitate moderată de discriminare pentru NLR (AUC=0,707) și PLR (AUC=0,709). Ceilalți markeri au prezentat o valoare diagnostică limitată.

Concluzii NLR și PLR oferă informații suplimentare privind inflamația sistemică asociată colestazei intrahepatice de sarcină și pot servi drept instrumente complementare în evaluarea clinică a acestor paciente. Sunt necesare studii suplimentare pentru confirmarea relevanței lor diagnostice și prognostice.

Cuvinte-cheie Colestaza intrahepatică de sarcină, colestaza gravidarum, CIS, markeri hematologici de inflamație.

INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP) is a hepatic disorder affecting bile flow that typically presents during the second and third trimesters of pregnancy. It is characterized by elevated serum bile acid (BA) levels and intense pruritus, usually affecting the palms and soles (1, 2). The diagnosis of ICP is based on serum BA levels ≥19 μmol/L, with levels ≥40 μmol/L indicating moderate disease and ≥100 μmol/L indicating severe intrahepatic cholestasis of pregnancy. This classification reflects the increased risk of adverse maternal and fetal outcomes, including preterm birth, fetal distress, and stillbirth, as BA levels rise (1-6). Although ICP symptoms and biochemical indicators generally resolve postpartum, the condition poses significant risks during pregnancy, particularly with respect to coagulation, fetal well-being, and the timing of delivery (7-9).

The pathophysiology of ICP remains incompletely understood, with evidence suggesting interactions among genetic, hormonal, and environmental factors (10-13). Genetic mutations affecting bile acid transport proteins, particularly in the ABCB4 and ABCB11 genes, have been associated with increased susceptibility to ICP, as these proteins play essential roles in bile acid transport and metabolism (11, 12, 14). Elevated estrogen and progesterone levels are also thought to exacerbate bile flow impairment, thereby increasing BA levels (7, 11). In addition, emerging evidence suggests that gut microbiota–bile acid–FXR signaling may influence bile acid homeostasis and inflammatory responses in ICP (11-15).

Given the potential inflammatory state in ICP, there is growing interest in hematological inflammatory markers, such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and mean platelet volume (MPV), as potential indicators of disease severity and progression (16-21). These markers are already well established in cardiovascular, autoimmune, and inflammatory conditions and are inexpensive and widely available in clinical practice (22-29). Studies indicate that elevated NLR and PLR values correlate with systemic inflammation and predict adverse outcomes in hepatic disorders, suggesting their potential utility in ICP (23-25, 30-35). Recent studies have also introduced novel biomarkers as promising tools for diagnosis and prognosis. These include the platelet-to-monocyte ratio (PMR), neutrophil-to-monocyte ratio (NMR), monocyte-to-lymphocyte ratio (MLR), eosinophil-to-lymphocyte ratio (ELR), and C-reactive protein-to-lymphocyte ratio (CLR) (26, 36, 37).

Hematological markers are increasingly recognized for their role in pregnancy complications. The NLR, calculated by dividing the neutrophil count by the lymphocyte count, has shown promise as a predictor of inflammation severity. In cardiovascular settings, higher NLR values are associated with worse outcomes (38, 39). Similarly, PLR, a ratio of platelet to lymphocyte counts, has demonstrated diagnostic value in systemic inflammation and is associated with adverse outcomes when elevated. Higher PLR values may indicate increased platelet activation, which is pertinent to ICP given the role of platelets in coagulation (33, 34). MPV, a measure of platelet size, has also been associated with heightened inflammatory responses and could be an indicator of coagulation risks in ICP, particularly given the coagulation challenges posed by possible Vitamin K deficiency in this population (40-42), although MPV's diagnostic value varies substantially across clinical contexts (29, 43, 44). These findings led the authors to examine their relevance in ICP.

Although guidelines from the Royal College of Obstetricians and Gynaecologists currently focus on monitoring bile acid levels and liver function in ICP, recent studies suggest that adding hematological inflammatory markers to routine evaluations could enhance ICP management by identifying women at higher risk of complications, such as hemorrhage, and providing additional clinical information, especially in settings where bile acid testing may be delayed or

less accessible (1-4). Ongoing research emphasizes that, when combined with biochemical markers, hematological indices such as NLR, PLR, and MPV could improve risk stratification and provide a more comprehensive clinical assessment of ICP (16). Integrating these markers may enable earlier identification of at-risk patients, thereby improving maternal and fetal outcomes in ICP management.

The aim of this study was to evaluate the diagnostic performance of hematological inflammatory markers in women with intrahepatic cholestasis of pregnancy and to determine their potential utility as complementary diagnostic biomarkers.

MATERIALS AND METHODS

Study Design and Participants

Participants were selected based on predefined inclusion and exclusion criteria. The study group included pregnant women diagnosed with intrahepatic cholestasis of pregnancy. The control group consisted of pregnant women matched for age and gestational week, without ICP. Exclusion criteria included pre-existing liver disease, preeclampsia, HELLP syndrome, and any known hematological disorders.

Sample Size Calculation

This study was designed as a case-control study aimed at evaluating the diagnostic performance of hematological inflammatory markers in intrahepatic cholestasis of pregnancy.

Data were collected between 2020 and 2023.

The sample size was calculated using Epi Info version 7.2.2.6 (StatCalc module, Sample Size and Power) for an analytical observational cohort study. The calculation was based on:

- a 95% confidence level;
- 80% statistical power;
- an expected 20% difference in the proportion of elevated inflammatory markers between the ICP and control groups, based on the literature (45, 46); and
- a 1:1 ratio between study groups.

The minimum required sample size was estimated at 44 participants per group. After accounting for a 10% non-response rate, this number increased to 48 participants per group.

To enhance statistical robustness, a total of 142 pregnant women were ultimately included and allocated to two groups according to predefined inclusion and exclusion criteria. The study group (L1) consisted of 71 women diagnosed with ICP, while the control group (L0) included 71 healthy pregnant women without ICP.

Data Collection and Laboratory Assessments

Participants were evaluated using questionnaires, medical record analysis, and laboratory tests, which included inflammatory markers such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), mean platelet volume (MPV), red cell distribution width (RDW), platelet-to-monocyte ratio (PMR), neutrophil-to-monocyte ratio (NMR), monocyte-to-lymphocyte ratio (MLR), eosinophil-to-lymphocyte ratio (ELR), C-reactive protein (CRP), and the CRP-to-lymphocyte ratio (CLR).

Calculation of Hematological Ratios

The NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count, while the PLR was calculated by dividing the absolute platelet count by the absolute lymphocyte count. Similarly, the PMR, MLR, NMR, ELR, and CLR were calculated by dividing the corresponding absolute blood cell counts. MPV and RDW values were automatically calculated using the Sysmex XN-1000 hematology analyzer.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 21 and GraphPad software. The normality of data distribution was assessed using the Shapiro–Wilk test. Continuous variables were presented as means and standard deviations (M ± SD) or medians and interquartile ranges (Me: Q1: Q3), depending on data distribution. Categorical variables were compared using the chi-square (χ^2) test with Yates' correction. A p-value < 0.05 was considered statistically significant, and 95% confidence interval (CI) was calculated.

The study was designed to evaluate the diagnostic performance of hematological inflammatory markers by comparing their levels between the ICP and control groups and assessing their discriminatory ability using receiver operating characteristic (ROC) curve analysis, to determine sensitivity (Se), specificity (Sp), and optimal cut-off values. The Youden index (J = sensitivity + specificity – 1) was applied to identify the optimal cut-off value for each hematological marker by maximizing the combined sensitivity and specificity derived from ROC curve analysis. Area under the ROC curve (AUC) values were categorized as follows: AUC > 0.9 (excellent), 0.81-0.9 (very good), 0.71-0.8 (good), and 0.61-0.7 (satisfactory). To increase statistical robustness and improve the precision of ROC estimates, a larger sample size of 71 participants per group was ultimately included.

RESULTS

The participants ranged in age from 18 to 43 years, with a mean age of 29.5 ± 6.3 years in L1 and 27.3 ± 5.4 years in L0 (p = 0.029).

Hemoglobin levels were assessed (Me 117: 106; 123) in the ICP group (L1), compared to (Me 118 (108; 124)) in the control group (L0). The average red blood cell count was (Me 3.8: 3.5; 4.2) in the ICP group vs. (Me 3.9: 3.6; 4.1) in the control group. The median hematocrit was (Me 36: 32.4; 37.9) in L1 vs. (Me 36: 33.7; 37.9) in L0.

Considering the high prevalence of anemia during pregnancy worldwide and its potential health risks for the mother and child, we analyzed data in terms of the presence and degree of anemia in the participants.

Pregnant women from both groups were categorized as follows, based on the presence and degree of anemia:

- Mild anemia (hemoglobin levels 100-109 g/L): L1 – 15 women (21.1%; 95% CI: 9.4-31.4%) vs. L0 – 12 cases (16.9%; 95% CI: 8.2-25.4%), χ^2 0.183; p=0.6689.
- Moderate anemia (hemoglobin levels 70-99 g/L): L1 – 11 women (15.5%; 95% CI: 6.6-25.4%) vs. L0 – 10 cases (14.1%; 95% CI: 5.6-22.8%), χ^2 0.056; p=0.8131.
- Severe anemia (hemoglobin levels <70 g/L) was not observed in either group.

The median platelet count was (Me 243: 209; 275) in L1 vs. (Me 230: 190; 278) in L0. The median neutrophil count was (Me 8.7: 6.9; 10.9) in L1 compared to (Me 8.0: 6.1; 10.5) in L0. The median lymphocyte count was (Me 1.7: 1.4; 2.1) in L1 vs. (Me 2: 1.7; 2.6) in L0.

The median NLR was (Me 5.1: 3.7; 7.2) in L1, compared to (Me 3.9: 3.0; 4.7) in L0. The median PLR was (Me 135.1: 110.5; 184.3) in L1 compared to (Me 104.4: 83.7; 132.9) in L0. The median MPV was (Me 11.8: 11.0; 12.6) fl in L1 and (Me 11.6: 10.9; 12.3) fl in L0. The median leukocyte count was (Me 11.8: 9.6; 14.5) $x10^3$/µL in L1 compared to (Me 9.3: 8.2; 11.4) $x10^3$/µL in L0.

Regarding the red cell distribution width indices, our findings revealed the following median values: RDW-SD: (Me 45.4: 41.4; 49.5) fl in L1 vs. (Me 48.8: 44.9; 53.1) fl in L0; RDW-CV: (Me 13.4: 12.8; 14.6) % in L1 vs. (Me 14.5: 13.6; 15.3) % in L0.

The median PMR was (Me 355.1: 285.9; 465.0) in L1, compared to (Me 305.4: 245.7; 391.1) in L0. The median NMR was (Me 11.1: 8.4; 13.5) in L1 compared to (Me 10.5: 8.6; 14.2) in L0. The median MLR was (Me 0.37: 0.30; 0.47) in L1 and (Me 0.4: 0.28; 0.52) in L0. The median ELR level was (Me 0.032: 0.024; 0.055) in L1 compared to (Me 0.036: 0.024; 0.057) in L0.

The median CRP level was (Me 4.1: 2.2; 8.2) mg/L in L1, compared to (Me 4.3: 1.8; 6.3) mg/L in L0. Elevated CRP levels were observed in 19 cases (26.8%; 95% CI: 16.9-34.7%) in L1 and 14 cases (19.7%; 95% CI: 11.0-31.2%) in L0, with no significant difference (χ^2 0.632; p=0.4268). The median CLR was (Me 1.75: 0.68; 3.09) in L1, compared to (Me 2.8: 1.41; 7.22) in L0.

Additionally, we evaluated hematological inflammatory markers through ROC curve analysis and determined their sensitivity and specificity (Table 1, Figure 1).

Table 1. Sensitivity and specificity of the hematological inflammatory markers in the study participants.

	AUC ROC	95% CI	p	Youden index	Associated criterion	Se (%)	Sp (%)
NLR	0.707	0.624-0.780	<0.0001	0.3803	>4.56	63.4	74.6
PLR	0.709	0.627-0.782	<0.0001	0.3099	>104.4	80.3	50.7
MPV	0.529	0.444-0.613	0.5509	0.09859	>11.9 fl	47.9	61.9
RDW-SD	0.648	0.564-0.727	0.0013	0.2394	≤45.4 fl	50.7	73.2
RDW-CV	0.663	0.579-0.740	0.0005	0.3099	≤13.7%	60.6	70.4
CRP	0.534	0.448-0.618	0.4853	0.09859	>6.1 mg/L	36.6	73.2
PMR	0.612	0.527-0.693	0.0176	0.1972	>245.71	94.37	25.35
NMR	0.520	0.434-0.604	0.6874	0.1127	≤15,8	90.14	21.13
MLR	0.551	0.466-0.635	0.2944	0.1549	≤0.5	87.32	28.17
ELR	0.502	0.417-0.587	0.9655	0.05634	≤0.12	88.73	5.63
CLR	0.624	0.538-0.703	0.0091	0.2394	≤4.39	87.32	36.62
Leucocytes	0.698	0.616-0.772	<0.0001	0.3380	>9.41 $x10^3$/µL	78.9	54.9

Note: NLR: neutrophil-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio, MPV: mean platelet volume, RDW-SD: red cell distribution width-standard deviation, RDW-CV: red cell distribution width-coefficient of variation, CRP: C-reactive protein, PMR: platelet-to-monocyte ratio, NMR: neutrophil-to-monocyte ratio, MLR: monocyte-to-lymphocyte ratio, ELR: eosinophil-to-lymphocyte ratio, CLR: CRP-to-lymphocyte ratio. ROC: receiver operating characteristic; AUC: area under the curve; CI: confidence interval; Se: sensitivity; Sp: specificity; p: probability value; fl: femtoliters; mg/L: milligrams per liter; µL: microliter.

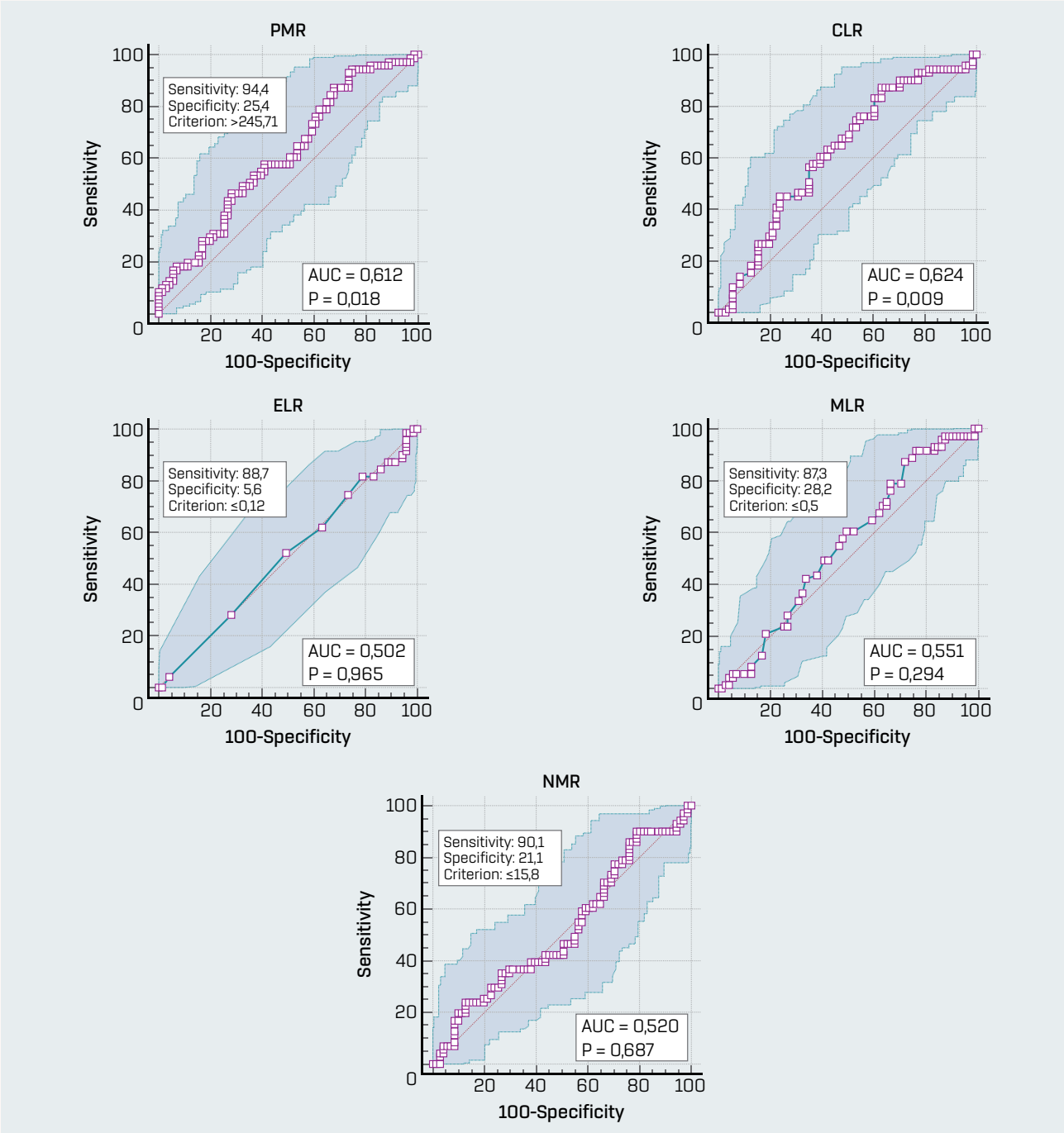

Figure 1. ROC curves of the hematological inflammatory markers in the study participants.

Hence, based on ROC analysis, the diagnostic value of NLR, PLR, RDW-SD, RDW-CV, PMR, CLR, and leukocyte levels for the diagnosis of ICP was considered satisfactory, with AUC values ranging from 0.61 to 0.70.

This study has several limitations that should be addressed in future research. The relatively small sample size may limit the generalizability of the findings, and the lack of long-term follow-up precludes an assessment of how inflammatory markers predict maternal and fetal outcomes beyond delivery. Larger, multicenter studies are needed to validate these findings and explore the role of novel markers such as CLR and PMR in ICP.

DISCUSSION

Principal Findings

The findings of this study highlight the potential role of hematological inflammatory markers, specifically the NLR and PLR, in the assessment and management of intrahepatic cholestasis of pregnancy. This study demonstrated that NLR and PLR are significantly elevated in pregnant women with ICP compared to healthy controls, and ROC analysis confirmed the satisfactory diagnostic performance of both markers. In contrast, other markers, including MPV, RDW, and newer ratios such as PMR and CLR, showed limited to moderate diagnostic utility. These low-cost, readily accessible markers proved useful for identifying ICP, with significantly higher levels observed in affected women.

Results in the Context of What is Known

Previous studies have proposed NLR and PLR as useful inflammatory indicators in hepatic, cardiovascular, autoimmune, and obstetric conditions (22, 23, 24, 25, 33, 34, 35). The elevated NLR and PLR levels observed in the ICP group are consistent with previous research highlighting the inflammatory nature of ICP. A study by Zhao et al. demonstrated that elevated NLR and PLR levels are indicative of systemic inflammation, which has been implicated in various hepatic disorders (34). Similarly, Liu et al. found that these markers are significantly associated with hepatic dysfunction and may serve as indicators of disease severity in liver-related conditions (33). Our study corroborates these findings, showing that both NLR and PLR had satisfactory areas under the curve in ROC analyses, with values of 0.707 and 0.709, respectively, reflecting the systemic inflammatory response associated with ICP.

The growing interest in hematological inflammatory markers in obstetric practice extends beyond intrahepatic cholestasis of pregnancy. Recent evidence suggests that NLR, PLR, and other composite inflammatory indices may have potential value in identifying women at increased risk of several pregnancy-related complications. Yuce et al. reported that elevated NLR and PLR values were associated with spontaneous preterm birth, supporting the role of systemic inflammation in adverse obstetric outcomes (47). Similarly, a systematic review and meta-analysis by Qiang et al. demonstrated that PLR may serve as a predictive marker for preeclampsia, highlighting its potential utility in risk assessment during pregnancy (48). In addition, Cui et al. found that elevated NLR values were associated with liver and coagulation dysfunction in women with preeclampsia, suggesting that inflammatory cell ratios may reflect the underlying pathophysiological processes affecting both the hepatic and hematologic systems (49). More recently, Kapci et al. reported that the systemic immune-inflammation index (SII) showed prognostic value in the diagnosis of preeclampsia, further supporting the concept that readily available blood count-derived inflammatory markers may provide clinically relevant information in obstetric disorders characterized by systemic inflammatory activation (50). Collectively, these findings reinforce the rationale for investigating hematological inflammatory markers in ICP and support their potential role as adjunctive indicators of disease-related inflammatory status.

The relatively modest AUC values observed in this study may be explained by the physiological immunological adaptations that occur during pregnancy, including baseline leukocytosis and altered inflammatory responses, which can reduce the discriminatory performance of systemic inflammatory markers.

The increased inflammatory response observed in patients with ICP can be attributed to the pathophysiological mechanisms underlying the disease. Elevated bile acid levels, a hallmark of ICP, have been shown to activate inflammatory pathways, contributing to systemic inflammation and oxidative stress (39).

This inflammatory cascade may explain the significant elevation in NLR and PLR levels observed in the present study.

While NLR and PLR demonstrated diagnostic potential, other markers, such as mean platelet volume (MPV) and red cell distribution width (RDW), were less informative. Similar findings were reported by Budzianowski et al., who noted that MPV often has variable diagnostic value across different conditions, depending on the disease context (29). Additionally, although RDW values were slightly lower in patients with ICP, the difference was not statistically significant, suggesting that RDW has limited utility in the clinical assessment of ICP.

Novel markers such as the platelet-to-monocyte ratio (PMR) and C-reactive protein-to-lymphocyte ratio (CLR) have shown promise in other inflammatory conditions but were not as strongly associated with ICP in this study. A recent investigation by Rezaeian et al. highlighted the potential of these markers in inflammatory diseases such as COVID-19, suggesting their broader applicability across systemic inflammatory states (26). However, their role in ICP warrants further investigation, as their sensitivity and specificity were suboptimal in the current study.

The inclusion of hematological inflammatory markers, notably NLR and PLR, in the standard diagnostic and monitoring protocol for ICP may provide additional information to support clinical assessment. The Royal College of Obstetricians and Gynaecologists currently emphasizes bile acid levels as the primary diagnostic marker for ICP (1). However, incorporating NLR and PLR into this assessment could provide a more comprehensive picture of disease progression, especially in resource-limited settings where advanced biochemical testing may not be readily available.

The significant association between elevated inflammatory markers and the presence of ICP also raises concerns about the potential maternal and fetal complications associated with systemic inflammation. Inflammatory markers have been linked to an increased risk of adverse outcomes, such as preterm birth and postpartum hemorrhage, emphasizing the need for proactive management strategies in patients with ICP (7).

Clinical Implications

The clinical utility of NLR and PLR lies in their accessibility, affordability, and potential to aid in the early diagnosis or risk stratification in ICP, particularly in settings where bile acid testing is delayed. Incorporating these markers into routine antenatal screening may assist clinicians in identifying women at higher risk of complications such as preterm birth or postpartum hemorrhage. Nevertheless, these markers should complement, rather than replace, standard diagnostic criteria.

Research Implications

Further research is needed to evaluate the prognostic value of NLR and PLR across diverse populations and clinical settings. Longitudinal studies should assess whether these markers are associated with maternal or neonatal outcomes, disease progression, or response to treatment. Additionally, further research is needed to explore and validate novel markers such as PMR and CLR in the context of ICP.

Strengths and Limitations

Strengths of this study include its design, clearly defined inclusion criteria, and the use of standardized hematologic analyses. However, its limitations include the relatively small sample size and single-center design, which

may limit the generalizability of the findings. The study also lacked long-term follow-up data to assess how these markers relate to clinical outcomes beyond delivery.

CONCLUSIONS

This study highlights the potential of NLR and PLR as accessible and cost-effective markers of systemic inflammation in intrahepatic cholestasis of pregnancy. The integration of NLR and PLR values into clinical practice could complement traditional diagnostic tools and improve the management of this complex condition. However, further research is required to validate these findings, explore the role of additional markers, and improve both diagnostic accuracy and patient-specific risk stratification in ICP.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

This case-control study was approved by the Research Ethics Committee of Nicolae Testemițanu State University of Medicine and Pharmacy (No. 46, dated 28 February 2020), and written informed consent was obtained from all participants prior to enrollment. The study was registered under ISRCTN21187408 https://www.isrctn.com/ISRCTN21187408

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PERCEIVED ETHICAL COMPETENCIES IN DOCTORAL RESEARCH IN THE HEALTH FIELD

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ABSTRACT

Introduction Ethical competencies of researchers contribute to ensuring scientific integrity, professional responsibility, and the protection of study participants. The aim of this study was to assess the perceived ethical competencies of doctoral students conducting research in the health field.

Materials and methods This paper presents a comprehensive cross-sectional study. Out of the total number of doctoral students (N=416), 274 participants completed the online questionnaire (response rate: 65.9%). The study questionnaire contained statements related to ethical competencies, self-assessed on a Likert scale. Data analysis included descriptive statistics and association tests (χ^2, t-test, Mann–Whitney).

Results The overall mean score of perceived ethical competencies was 4.01, indicating a high level of confidence in respondents' own abilities. The highest scores were reported for competencies related to protecting the confidentiality of participants' data, honest reporting of research results, and obtaining informed consent. Participants who had received training in research ethics reported significantly higher mean scores compared to those without such training ($p < 0.05$).

Conclusions Doctoral students report a high level of perceived ethical competencies; however, gaps remain in the areas of regulatory framework knowledge and methodological aspects of research, suggesting the need to strengthen targeted training in research ethics.

Keywords Ethical competencies, doctoral students, doctoral education, biomedical research.

COMPETENȚE ETICE PERCEPUTE ÎN CERCETAREA DOCTORALĂ DIN DOMENIUL SĂNĂTĂȚII

Introducere Competențele etice ale cercetătorilor contribuie la asigurarea integrității științifice, a responsabilității profesionale și a protecției participanților la studii. Scopul studiului constă în evaluarea competențelor etice ale doctoranzilor care realizează cercetări în domeniul sănătății.

Materiale și metode Prezenta lucrare reprezintă un studiu transversal integral. Din numărul total de doctoranzi (N=416), 274 de persoane au completat chestionarul, distribuit online (rata de răspuns 65,9%). Chestionarul studiului a conținut afirmații privind competențele etice, autoevaluate pe o scală Likert. Analiza datelor a inclus statistici descriptive și teste de asociere (χ^2, t-test, Mann–Whitney).

Rezultate Scorul mediu general al competențelor etice percepute este de 4,01, indicând un nivel ridicat de încredere în propriile abilități. Cele mai mari scoruri au fost raportate pentru competențele privind protejarea confidențialității datelor, prezentarea onestă a rezultatelor și obținerea consimțământului informat. Participanții care au beneficiat de instruire în etica cercetării au înregistrat scoruri medii semnificativ mai mari comparativ cu cei neinstruiți ($p<0,05$).

Concluzii Doctoranzii raportează un nivel ridicat al competențelor etice percepute, însă există lacune privind cunoașterea cadrului normativ și a aspectelor metodologice în cercetare, ceea ce sugerează necesitatea consolidării formării punctuale în etica cercetării.

Cuvinte-cheie Competențe etice, doctoranzi, studii de doctorat, cercetare biomedicală.

INTRODUCTION

Researchers' ethical competencies are a fundamental component of ethical research management, ensuring adherence to the principles of integrity and professional responsibility in the conduct of scientific studies. Developing and strengthening researchers' ethical competencies throughout their professional careers is an important goal for institutions with health research units. It is important for ethics education to begin at the start of a research career. Today, doctoral schools in the biomedical sciences worldwide design and implement comprehensive curricula in which advanced theoretical knowledge and practical professional skills are developed alongside researchers' ethical competencies. In the field of health, ethical competencies are of particular importance, as studies can have a direct impact on people's lives and health. Without these competencies, research risks producing distorted results, damaging the reputation of institutions, and endangering study participants or the communities being studied (1).

The concept of ethical competence has been defined by several authors (2; 3), but in most cases the concept refers to knowledge and attitudes that encourage certain moral practices or behaviors. In the context of research ethics, ethical competence can be understood as a set of knowledge, skills, and attitudes that enable researchers to identify ethical dilemmas and apply ethical principles and norms in the design and conduct of studies at all stages of research (4). Ethical competence involves a combination of theoretical knowledge, practical skills, and attitudes that enable researchers to make sound and responsible decisions in complex situations, ensuring the integrity of the scientific process and the protection of study participants (5). Ethical competencies enable researchers to contribute to evidence-based research practices, influence clinical practice, and strengthen public trust in science. Based on the above, the development of ethical competencies in health research is a strategic necessity for any institution with research units.

Research ethics training programs are developed and implemented globally, often with the aim of strengthening local capacity to ensure ethical conduct in health-related research. At the same time, international research findings suggest that research ethics education programs have become more effective over the past decade, contributing to improvements in participants' knowledge, skills, and ethical attitudes. The greatest impact is achieved when training uses active teaching methods, involves multiple instructors with complementary expertise, incorporates case studies, and provides practical opportunities to apply ethical principles. (6; 7) In the Republic of Moldova, doctoral education in the field of health has been in the process of adapting to international standards for more than a decade. There is a curriculum in research ethics and methodology, but no study has been conducted on the level of ethical competence among doctoral students.

The objective of this study was to assess the perceived ethical competence of doctoral students conducting research in the health field. While perceived ethical competence does not necessarily correspond to objectively measured competence, self-assessment provides valuable insight into researchers' confidence in applying ethical principles, identifies perceived educational needs, and informs the development of targeted training interventions. Self-perceived competence is widely recognized in educational and professional development research as an important determinant of motivation, engagement, self-regulated learning, and professional behavior (8).

MATERIALS AND METHODS

Study Design

To achieve the stated objective, a cross-sectional study was conducted – a design frequently used in educational, social, and biomedical research because it provides an overview of the phenomenon under study and facilitates comparative analysis among subgroups, especially when similar studies are lacking (9; 10).

Study Population and Sample

The target population of the study consisted of doctoral students in the Doctoral School of Medical Sciences. The entire active population of doctoral students, comprising 416 individuals (data provided by the Doctoral School office in February 2025), was invited to participate in the study. A total of 274 individuals actually participated, resulting in a response rate of 65.9%, which is considered adequate for online surveys (11).

Data Collection Instrument

The data collection tool is a questionnaire consisting of 11 questions designed to collect sociodemographic data and 15 closed-ended questions designed to assess the respondents' perceived ethical competencies (the items were developed based on international ethical standards in biomedical research, such as the provisions of the Declaration of Helsinki (12). The conceptual framework of the questionnaire consisted of statements with responses on a 5-point Likert scale. For each statement, respondents rated their own level of competence on a scale from 1 to 5, where 1 means *not at all prepared* and 5 means *fully prepared*. It should be noted that the questionnaire used in this study is a subsection of a broader research instrument, developed and validated as part of the research project "Ethical Management of Health Research." (13)

Data Collection Procedure

The study data were collected online using the Google Forms platform.

Statistical Analysis

For each item in the questionnaire, mean scores (including standard deviations) were calculated, and the competencies with the lowest and highest mean values were highlighted. The use of means for ordinal Likert-type data can be considered an acceptable approximation in studies with moderate sample sizes (14). In this study, means and standard deviations were used for descriptive purposes to facilitate interpretation and comparison across items, an approach frequently applied in educational research and in the assessment of self-reported competencies (15). At the same time, combining these with nonparametric methods allowed for a robust interpretation of the results. The literature also supports the view that aggregate scores derived from multiple items measuring the same construct can be analyzed using parametric statistics, particularly in the case of moderate- or large-sized samples (14, 16). The competencies with the lowest reported mean scores were analyzed using nonparametric Chi-square (χ^2) association tests to determine their relationships with the variable "formal training in research ethics." The analysis of differences between groups (those trained vs. those not trained in research ethics) was conducted using both the t-test for independent samples and the nonparametric Mann–Whitney U test, which is appropriate for ordinal data, to validate the robustness of the results. The effect size for the nonparametric tests was estimated using the r coefficient ($r = Z/\sqrt{N}$). All statistical analyses were performed using Excel (for Microsoft 365) with the Real Statistics add-in, with a significance level set at $p < 0,05$.

RESULTS

An analysis of the respondents' sociodemographic characteristics reveals a predominantly female profile, with women accounting for 72.6% of the sample (95% CI: 67.1%–77.6%), with a median age of 35 years and an interquartile range (IQR) of 32–39 years. In terms of career trajectory, most respondents are in the early stages of their research careers, with 73.4% reporting 0–5 years of experience in scientific work (95% CI: 67.8%–78.5%). Regarding training in research ethics, 72.6% of participants (95% CI: 67.1%–77.6%) stated that they had previously received specific training, which was primarily delivered through formal teaching sessions (courses or institutional academic modules).

The ethical competencies assessed in the study were those related to: 1) knowledge of and application of the principles of the Declaration of Helsinki in research; 2) knowledge and application of national regulations and legislation governing research involving human participants; 3) competencies in formulating feasible study objectives and a valid methodology; 4) adherence to the principle of fairness in recruiting research participants; 5) identifying and minimizing risks to study participants; 6) providing accurate and complete information to participants and obtaining informed consent; 7) protecting the confidentiality of study participants' data; 8) preparing the application and submitting it to the research ethics committee; 9) seeking advice from colleagues or the ethics committee in situations involving ethical dilemmas; 10) reporting issues related to research ethics and integrity; 11) honestly presenting research results; 12) citing sources correctly and avoiding plagiarism in scientific publications; 13) applying ethical principles of authorship in research; 14) managing conflicts of interest in research; 15) effectively managing data in the study.

As a result of the descriptive analysis of all assessed perceived competencies, an average score of 4.01 points was found, which demonstrates the respondents' increased confidence in their own ethical skills in research. The highest scores were recorded for the following competencies: *Knowledge of and adherence to the principle of protecting the confidentiality of study participants' data* (M = 4.28, SD = 0.81); *Honest and complete presentation of research results* (M = 4.22, SD = 0.85); *Accurate and complete information provided to study participants and obtaining valid informed consent* (M = 4.20, SD = 0.82). The lowest scores were recorded for the following competencies: *Knowledge of and application of the principles of the Declaration of Helsinki in research* (M = 3.71, SD = 0.95); *Knowledge and application of national regulations and legislation governing research involving human participants* (M=3.77, SD=0.92); *Formulation of feasible study objectives and valid methodology* (M=3.79, SD=0.87).

We hypothesized that the ethical skills with the lowest scores might be associated with the level of training in research ethics, and to test this hypothesis, we conducted bivariate association analyses between these variables. Thus, *regarding knowledge and application of the principles of the Declaration of Helsinki*, we found noticeable descriptive differences in the distribution of responses between those who had taken courses in research ethics and those who had not. Those who had taken courses tended to select scores of 4 and 5 (well-prepared and fully prepared) more frequently, while those without such training tended to select scores of 3 and 4 (moderately prepared and well-prepared) more frequently (Figure 1).

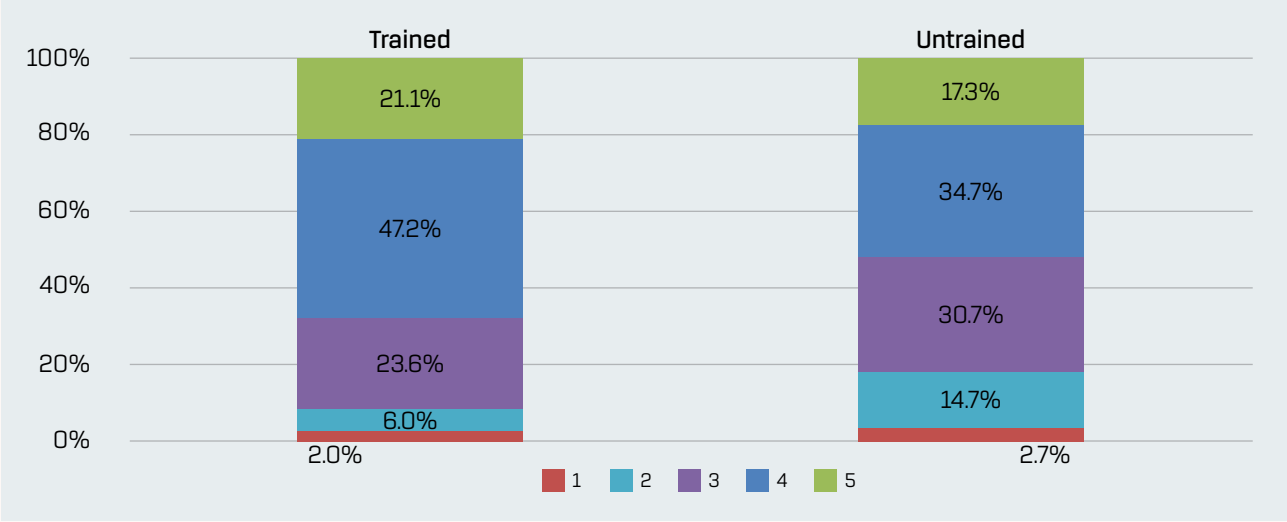

Figure 1. Distribution of responses regarding knowledge of and adherence to the Declaration of Helsinki in research, by level of training in research ethics (%).

However, application of the chi-square test did not reveal a statistically significant association between the variable "training in research ethics" and respondents' perceptions of their own level of preparedness in applying the Declaration of Helsinki ($\chi^2(4) = 8.36$, $p = 0.079$, Cramer's $V = 0.175$).

Regarding *knowledge and application of national regulations and legislation governing research involving human participants*, respondents with training in research ethics most frequently selected score 4 (43.7%), followed by 5 (25.6%) and 3 (25.1%); while very few selected a score of 1 (0.5%) or 2 (5%). In contrast, those without training in research ethics were more likely to select scores of 3 (34.7%) and 4 (36.0%); score 5 was selected comparatively less often (13.3%); and scores of 2 (13.3%) and 1 (2.7%) are selected more frequently in this group compared to those who have received training (Figure 2). Application of the chi-square test did not reveal a statistically significant association between these analyzed variables ($\chi^2(4) = 13.75$, $p = 0.08$, Cramer's $V = 0.11$).

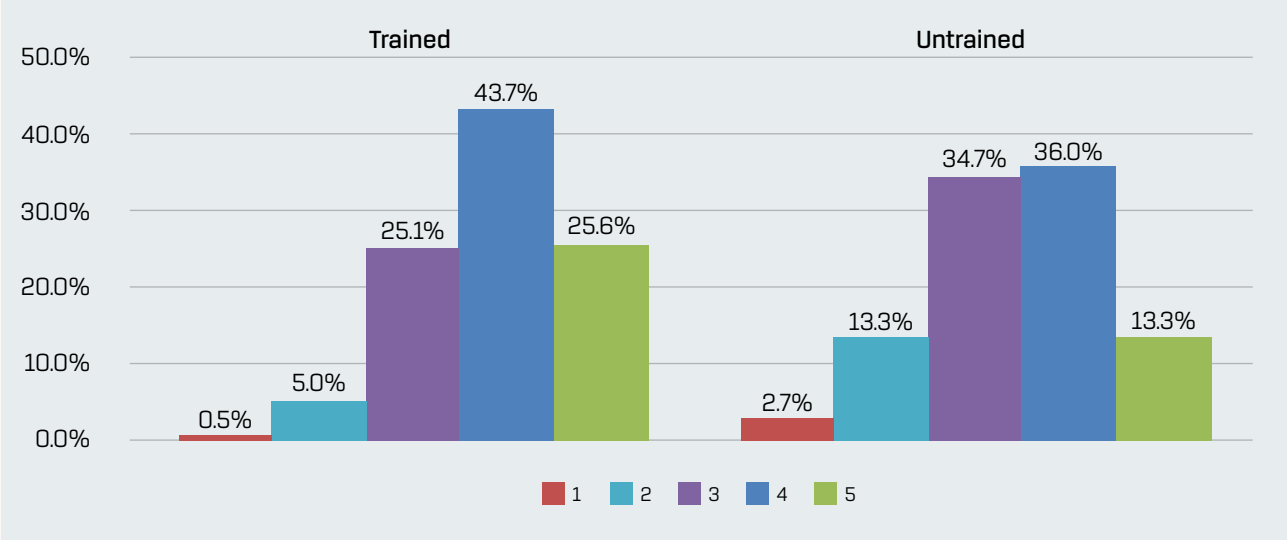

Figure 2. Distribution of responses regarding the application of national regulations and legislation in research, by level of training in research ethics (%).

A descriptive analysis of the differences between the *trained* and *untrained* groups, with regard to perceived competence in formulating feasible study objectives and valid methodology, reveals that scores of 4 and 3 predominate in both groups. Among those who *received training*, 47.7% gave a score of 4, and 24.1% gave a score of 3. In the group that *did not receive training*, 44.0% of respondents gave a score of 4, and 32.0% gave a score of 3. Those with training in research ethics more frequently select a score of 5 (21.6%) than those in the group without training – 16.0% (Figure 3). An analysis of the association between the variables using the chi-square test of independence did not reveal any statistically significant relationships ($\chi^2(4) = 2.47$, $p = 0.64$, Cramer V =0.09).

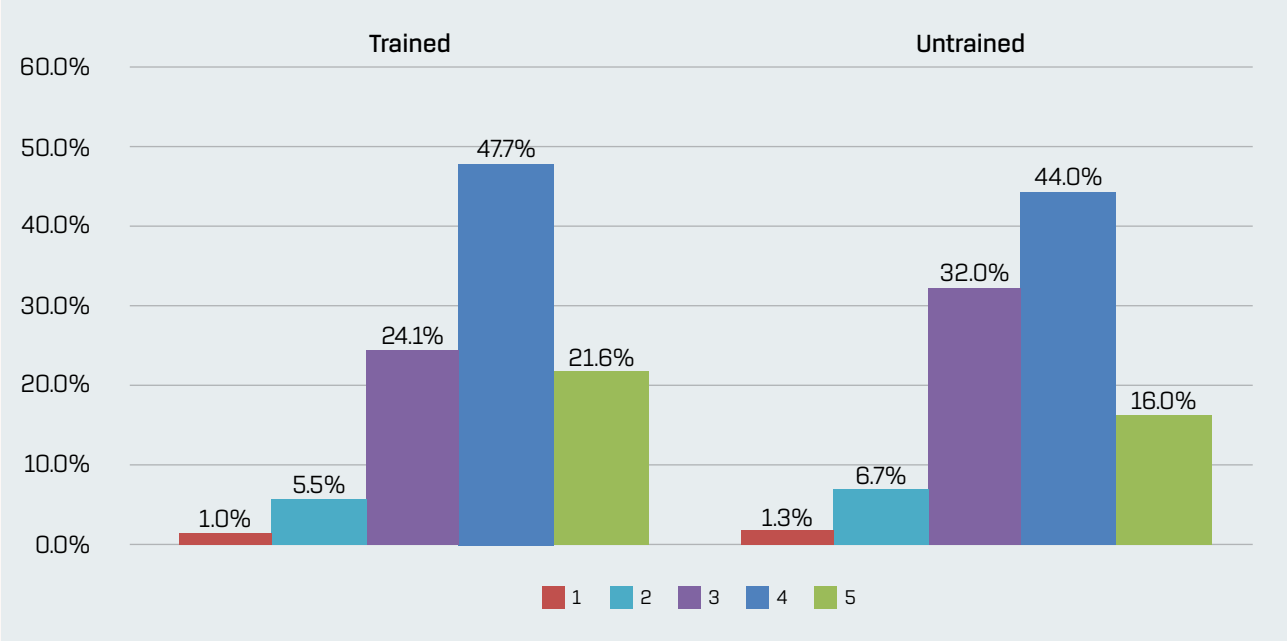

Figure 3. Distribution of responses regarding the formulation of feasible study objectives and valid research methodology, by level of training in research ethics (%).

The study also compared the average scores of the two groups – trained and untrained – for all 15 assessed competencies. Accordingly, by applying the *t-test for independent samples*, a statistically significant difference was found between the two groups ($t(274) = 2.29$, $p = 0.023$). The *trained* participants achieved slightly higher mean scores ($M = 4.06$) than the *untrained* participants ($M = 3.84$). The statistically significant difference was also confirmed by the nonparametric Mann–Whitney test ($U = 6553$, $p = 0.039$). The values of the *r* coefficient (calculated based on the Mann–Whitney test) range from 0.13 to 0.19, indicating that training in research ethics has a positive impact on perceived skills, but this impact is only small to moderate.

Thus, although the hypothesis that *formal training* in research ethics would positively influence the perceived competencies that received the lowest scores was not statistically confirmed, the overall findings (across all competencies assessed) indicate that training in research ethics contributes moderately to increased confidence and perceived competence, even though the impact on specific skills may be limited.

DISCUSSION

The results indicate that doctoral students believe they possess ethical competencies above the average (M=4.01, on a scale of 1 to 5), which reveals a high level of self-confidence. The differentiated analysis, however, reveals a divide between procedural competencies (*maintaining confidentiality*, obtaining *informed consent, and honest presentation of research results*), which appear to be well internalized, and normative-legal and methodological competencies, where confidence is lower. A high perception of competence can influence an individual's ability to effectively tackle complex tasks and maintain a high level of engagement and performance, according to self-determination theory (17). At the same time, a high level of self-perceived competence may suggest a *ceiling effect* and should be interpreted with caution, as it can be influenced by psychological factors such as a) social desirability bias, i.e., the tendency of individuals to respond to questionnaires in a socially acceptable manner, rather than truthfully, and/or positive self-deception (self-deceptive enhancement), whereby individuals sincerely perceive their competencies in a favorable light (18; 19). Furthermore, the literature shows that individuals with limited experience in a field may tend to overestimate their abilities. This phenomenon is known as the Dunning–Kruger effect and occurs when a lack of knowledge impairs the ability to make a realistic self-assessment of one's performance (20). Thus, the high level of perceived ethical competence identified among doctoral students may suggest an increased confidence in their ability to conduct research responsibly, even if this perception does not always reflect their actual level of competence. Therefore, further studies are needed to assess realistic ethical competencies in doctoral research in the field of health.

The analysis of the study data does not identify a statistically significant association regarding the impact of research ethics training on the level of confidence in the perceived competencies that received lower scores. However, this (statistically moderate) association is detected using the t-test for independent samples, comparing the overall mean scores of *trained* and *untrained* respondents, which confirms that research ethics education plays a beneficial role in increasing perceived confidence in ethical competencies in doctoral research. The fact that this association is only moderate highlights the need to strengthen the training component in research ethics for researchers. Several authors (21; 22) emphasize that in order to develop the ability to interpret and apply ethical rules in various contexts, research ethics training must include case studies, problem-based scenarios, mentoring, and interactive methods, while maintaining a connection to the basic norms and regulations in the field.

CONCLUSIONS

The study found an above-average level of confidence in doctoral students' own ethical competencies, which is significantly associated with training in research ethics. This finding is relevant in the context of self-determination theory, which considers the perception of competence to be an important resource for professional engagement and performance. At the same time, although formal training has a positive impact on self-assessment, this effect is only moderate and not strong enough to reduce perceived competence gaps in certain areas, such as knowledge of and application of international and national regulatory frameworks in research, including the ability to formulate feasible scientific objectives and valid methodologies. This imbalance suggests the need to strengthen theoretical and practical training on the international and national regulatory framework in research, as well as on the methodological design of research. At the same time, the high mean values

reported for several competencies may suggest the presence of a possible ceiling effect, characterized by a concentration of responses at the upper end of the assessment scale, which may limit data variability and the sensitivity of statistical analyses.

CONFLICT OF INTEREST

None declared.

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ETHICS APPROVAL AND PARTICIPANT CONSENT

The study was approved by the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy in the Republic of Moldova, Minutes No. 70, dated August 08, 2024. Implicit consent was obtained.

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CYSTIC ECHINOCOCCOSIS IN THE REPUBLIC OF MOLDOVA (2011-2024): EPIDEMIOLOGICAL CHARACTERISTICS AND THE IMPORTANCE OF THE ONE HEALTH APPROACH

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ABSTRACT

Introduction Cystic echinococcosis (CE) is a zoonotic parasitic disease that affects both human and animal health, causing considerable socioeconomic losses. This study aimed to assess the epidemiological trends and distribution of CE in humans and livestock in the Republic of Moldova from a One Health perspective.

Materials and methods A retrospective descriptive study was conducted using data from the National Agency for Public Health (NAPH) and the National Agency for Food Safety (NAFS). Human CE surgical cases reported during 2011–2024 (n=995) and CE cases detected in slaughtered animals during 2014–2023 (n=95,898) were analyzed. Indicators included incidence, demographic and territorial distribution, and cyst localization.

Results Human CE incidence decreased from 2.2 to 1.87 cases per 100,000 population during 2016–2024, while animal prevalence declined from 24.5% in 2014 to 4.7% in 2024. The highest incidence rates were recorded in Ceadîr-Lunga (12.6), Drochia (6.2), Taraclia (6.1), Florești and Basarabeasca (6.0), and Leova (5.7) per 100,000 population, exceeding the national average (1.9). Adults aged 31-60 years accounted for 59.0% of human cases. Sheep had the highest prevalence among livestock species (7.3%).

Conclusions Although declining trends were observed in both humans and animals, CE remains a significant public health and veterinary concern in the Republic of Moldova. Strengthened surveillance and intersectoral collaboration within the One Health framework are essential to reduce transmission and improve disease control.

Keywords Cystic echinococcosis, One Health, human health, farm animals.

ECHINOCOCOZA CHISTICĂ ÎN REPUBLICA MOLDOVA (2011-2024): CARACTERISTICI EPIDEMIOLOGICE ȘI IMPORTANȚA ABORDĂRII „O SINGURĂ SĂNĂTATE"

Introducere Echinococoza chistică (EC) este o boală parazitară zoonotică ce afectează atât sănătatea umană, cât și cea animală, generând pierderi socioeconomice considerabile. Studiul a avut ca scop evaluarea tendințelor epidemiologice și a distribuției EC la om și animale de fermă în Republica Moldova, din perspectiva conceptului *OneHealth*.

Materiale și metode A fost realizat un studiu retrospectiv descriptiv, utilizând date furnizate de Agenția Națională pentru Sănătate Publică (ANSP) și Agenția Națională pentru Siguranța Alimentelor (ANSA). Au fost analizate cazurile umane de EC confirmate chirurgical,raportate în perioada 2011–2024 (n=995) și cazurile de EC depistate la animalele sacrificate în perioada 2014–2023 (n=95.898). Indicatorii analizați au inclus incidența, distribuția demografică și teritorială, precum și localizarea chisturilor.

Rezultate Incidența EC la om a scăzut de la 2,2 la 1,87 cazuri la 100.000 populație în perioada 2016-2024, iar prevalența la animale s-a redus de la 24,5% în anul 2014 la 4,7% în anul 2024. Cele mai înalte rate de incidență au fost înregistrate în raioanele Ceadîr-Lunga (12,6), Drochia (6,2), Taraclia (6,1), Florești și Basarabeasca (6,0), precum și Leova (5,7) la 100.000 populație, depășind media națională de 1,9. Adulții cu vârsta cuprinsă între 31 și 60 de ani au reprezentat 59,0% din totalul cazurilor umane. Dintre speciile de animale, ovinele au prezentat cea mai înaltă prevalență a infecției (7,3%).

Concluzii Deși au fost observate tendințe descrescătoare atât la om, cât și la animale, echinococoza chistică rămâne o problemă importantă de sănătate publică și sănătate animală în Republica Moldova. Consolidarea supravegherii și a colaborării intersectoriale în cadrul abordării *OneHealth* este esențială pentru reducerea transmiterii și îmbunătățirea controlului bolii.

Cuvinte-cheie Echinococoză chistică, OneHealth, sănătate umană, animale de fermă.

INTRODUCTION

Echinococcosis is a zoonotic helminth infection caused by cestodes of the genus Echinococcus, affecting humans as well as domestic and wild animals and generating substantial public health, veterinary, and economic burdens (1–5). According to the World Health Organization (WHO), cystic echinococcosis (CE), caused by *Echinococcus granulosus sensu lato*, is classified as a neglected tropical disease and remains one of the most important foodborne parasitic infections worldwide (5).

The One Health approach, which recognizes the interconnectedness of human, animal, and environmental health, has become increasingly important for the prevention and control of CE. Recent international frameworks emphasize the need for integrated surveillance and coordinated interventions across human, animal, and environmental sectors to achieve sustainable control of zoonotic diseases, including cystic echinococcosis (6-8). Inadequate surveillance systems, insufficient control of definitive and intermediate hosts, and limited coordination between public health and veterinary sectors contribute to the persistence of transmission in endemic areas (5, 9-11).

Because of its progressive, often asymptomatic, but often with dramatic consequences that are difficult to detect and hardly detectable, echinococcosis is a typical example of a disease characterised by the „iceberg phenomenon" (1).

The two forms of echinococcosis of greatest public health importance are CE and alveolar echinococcosis (AE). CE is the most prevalent form globally and is associated with considerable morbidity and socioeconomic impact (2-5, 12). The genus *Echinococcus* currently includes several species of medical and veterinary importance, including *Echinococcus granulosus sensu lato, Echinococcus multilocularis, Echinococcus shiquicus, Echinococcus vogeli* and *Echinococcus oligarthrus* (13).

Echinococcoccusgranulosus has a worldwide distribution and is present on all inhabited continents except Antarctica. In contrast, AE is largely restricted to the Northern Hemisphere, particularly in parts of China, the Russian Federation, and several countries in Europe and North America (5).

The burden of CE extends beyond its direct health consequences. In livestock, infection results in the condemnation of affected organs, reduced productivity, decreased milk yield, impaired fertility, and economic losses for farmers and the livestock industry (1, 5, 12).

In the European Union (EU), 540 cases of echinococcosis were reported in 2021, corresponding to a notification rate of 0.16 cases per 100,000 population. In 2022, the number increased to 731 cases, with a notification rate of 0.19 per 100,000 population (14-16). A recent study by Casulli et al. reported 64,745 human CE cases across 40 European countries between 1997 and 2020, with an average annual incidence of 0.64 cases per 100,000 population (13). Recent European assessments have confirmed that, despite overall reductions in incidence, cystic echinococcosis remains an important public health concern in several countries of southeastern and eastern Europe, where surveillance and control activities continue to face important challenges (11, 13).

In the Republic of Moldova, CE remains an important public health concern. It ranks among the most frequently reported helminth infections and continues to cause significant health and economic consequences (17, 18). Annual fluctuations in reported cases may reflect both changes in transmission dynamics and improvements in diagnostic capabilities, including advances in imaging and laboratory investigations (19).

Human echinococcosis accounted for 0.5%–0.9% of all reported helminth infections during 2021–2024, with an average proportion of approximately 0.8% in the last two years of observation (19). Dogs (*Canis lupus familiaris*) serve as the principal definitive hosts of *E. granulosus* and play a central role in maintaining transmission as definitive hosts of *E. granulosus*, contaminating the environment through the excretion of parasite eggs (5, 14-16, 20-22).

Several factors contribute to the persistence of CE transmission in Moldova, growing livestock populations, particularly sheep, the presence of free-roaming and inadequately dewormed dogs, and insufficient control measures in rural settings (22). Recent global burden estimates indicate that CE continues to impose a substantial health and economic burden worldwide, particularly in endemic regions where transmission cycles remain active (23).

Due to its slow progression, chronic nature, and frequently asymptomatic clinical course, cystic echinococcosis is often diagnosed at a late stage in humans, while in livestock it is commonly detected only during routine post-mortem inspection at NAFS-authorized slaughterhouses.

The working hypothesis of the study was that cystic echinococcosis remains endemic in the Republic of Moldova despite ongoing control measures, as reflected by persistent occurrence in both human and animal populations and by heterogeneous geographical distribution patterns.

The aim of this study was to assess the epidemiological situation of human and animal cystic echinococcosis in the Republic of Moldova and to identify its temporal, demographic, and geographical characteristics within a One Health framework.

MATERIALS AND METHODS

Study Design

A retrospective descriptive observational study was conducted in the Republic of Moldova to assess the epidemiological trends and distribution of cystic echinococcosis (CE) in humans and livestock within a One Health framework. The study covered the period 2011–2024 for the human component and 2014–2024 for the veterinary surveillance component, while prevalence analyses requiring denominator data were restricted to 2014–2023.

Human Surveillance Data

For the human component, data on surgically confirmed cases of cystic echinococcosis reported by healthcare institutions to the National Agency for Public Health (NAPH) were analyzed. A total of 995 human cases registered between 2011 and 2024 were included. Cases were characterized according to age, sex, place of residence (urban/rural), administrative territory, cyst localization, and disease form (primary or recurrent). Data were extracted from the national epidemiological surveillance system using Form No. 1 – *Statistical Report on Parasitic Disease Morbidity*.

Human incidence rates were calculated per 100,000 population using official population estimates provided by the National Bureau of Statistics of the Republic of Moldova. Incidence was analyzed by year, age group (children and adults), place of residence, and administrative territory. The geographical distribution of cases was assessed at district and municipality levels to identify areas with increased disease incidence.

Veterinary Surveillance Data

Veterinary data were obtained from the National Agency for Food Safety (NAFS). Two complementary surveillance datasets were used. The first dataset comprised annual records of cystic echinococcosis cases detected during post-mortem inspection of slaughtered animals and was available for 2014–2024. The second dataset included the total number of animals slaughtered in NAFS-authorized slaughterhouses, stratified by species (cattle, sheep, pigs, and horses), and was available only for 2014–2023.

Since prevalence estimation requires both the number of infected animals and the total number examined, prevalence calculations and species-specific analyses were limited to the period 2014–2023. During this period, 4,169,323 animals were inspected, of which 95,898 were diagnosed with cystic echinococcosis. Annual prevalence was calculated as the proportion of infected animals among all examined animals and was further analyzed by animal species.

Data quality and comparability were considered acceptable because veterinary inspections were performed according to standardized veterinary-sanitary procedures.

Statistical Analysis

Temporal trends in human incidence and animal prevalence were evaluated using simple linear regression models. Pearson's correlation coefficient was calculated to assess the relationship between the annual number of human cases and animal cases during the period 2014–2024.

Data management and statistical analyses were performed using Microsoft Excel 2021. Human incidence rates were expressed per 100,000 population, whereas animal prevalence was expressed as the percentage of infected animals among those examined. Veterinary prevalence estimates were additionally interpreted according to animal species and annual slaughter volumes.

Ethical Considerations

The study was based exclusively on secondary, anonymized, aggregated surveillance data; therefore, informed consent was not required. Data processing complied with national regulations on confidentiality and personal data protection.

This study was conducted within the framework of the Field Epidemiology Training Program (FETP-Intermediate) of the Eastern Europe and Central Asia (EECA) Regional Office of the Centers for Disease Control and Prevention (CDC).

RESULTS

Surveillance and monitoring of CE in humans

Analysis of epidemiological surveillance data showed that the incidence of CE was consistently higher among adults than among children throughout the study period (2011–2024). In adults, incidence increased during the early years of observation, reaching a peak of 4.2 cases per 100,000 population in 2012–2013, followed by a gradual decline until 2016. A further decrease was observed in 2020, after which incidence increased again, reaching approximately 3.0 cases per 100,000 population in 2022–2023.

In contrast, incidence among children remained substantially lower during the entire study period. Rates increased from 0.9 cases per 100,000 population in 2011 to a peak of 2.5 cases per 100,000 population in 2014, followed by a marked decline to 0.7 cases per 100,000 population in 2016. From 2017 onwards, incidence remained very low, reaching nearly zero in 2020 and 2022. A slight increase was observed in 2024, when the incidence reached 0.4 cases per 100,000 population (Figure 1).

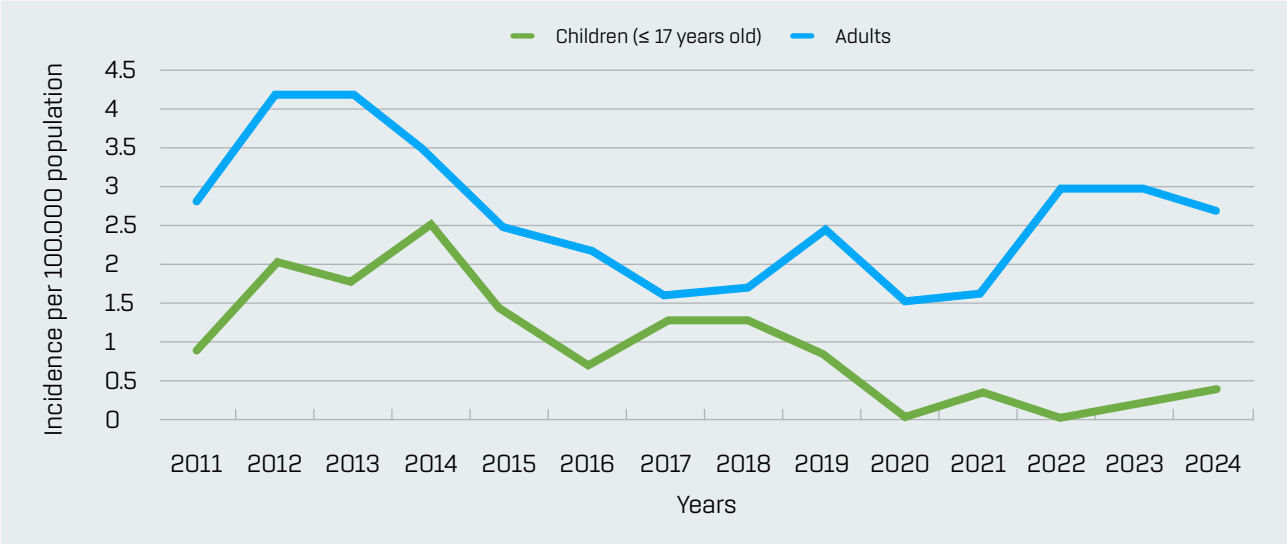

Figure 1. Incidence of echinococcosis by age group (children ≤17 years and adults), Republic of Moldova, 2011–2024.

The age distribution of cases showed that the most affected groups were individuals aged 51–60 years (23%), followed by 61–70 years (19%) and 41–50 years (17%). Overall, adults aged 31–60 years accounted for the majority of cases, representing 59% of all patients. The mean age of patients was 30 years.

No cases were recorded among children aged 0–2 years. Among children, the highest number of cases was observed in the 3–6-year age group (n = 9), representing 34.6% of all pediatric cases, while 17 cases were reported among children aged 7–17 years.

According to data reported to the NAPH, a total of 995 patients underwent surgical treatment for cystic echinococcosis in the Republic of Moldova between 2011 and 2024. Figure 2 illustrates the annual number of surgically confirmed cases recorded during the study period. The annual number of cases showed an overall decreasing trend between 2011 and 2020, reaching a peak of 149 cases in 2013 and declining to 32 cases in 2020, representing a reduction of more than fourfold over seven years. From 2021 onwards, the number of reported cases increased, reaching a secondary peak of 60 cases in 2023, followed by a slight decline to 45 cases in 2024.

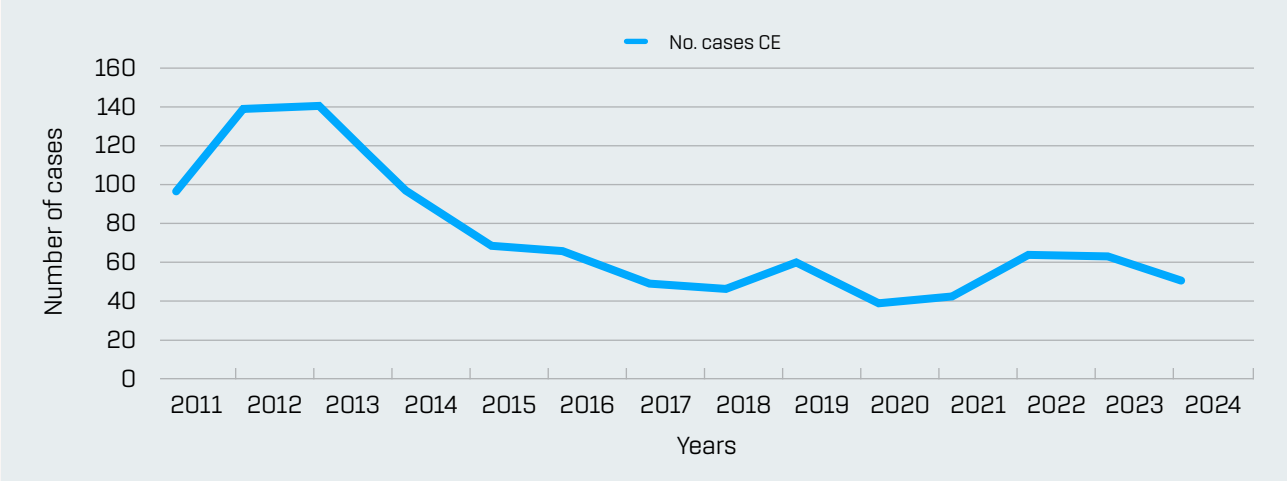

Figure 2. Distribution of CE cases in the human population, Moldova, 2011-2024, N = 995

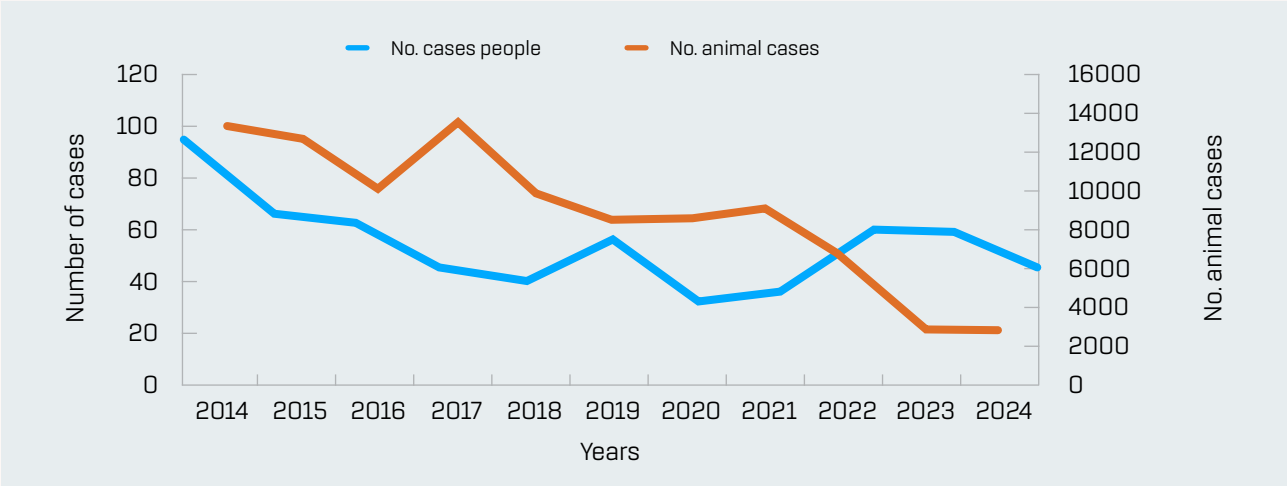

Figure 3. Distribution of CE cases in humans and livestock, Moldova, 2014-2024

Analysis of veterinary surveillance data revealed a substantial decline in the number of CE cases detected in slaughtered animals during 2014–2024. In 2014, 13,444 positive cases were identified, representing 24.5% of all animals examined, whereas in 2024, 2,820 positive cases were recorded, corresponding to a prevalence of 4.7%. Overall, the number of positive animal cases decreased approximately fivefold during the study period (Figure 3).

In contrast, the number of human CE cases showed greater temporal variation. Following a declining trend between 2014 and 2020, when the annual number of cases decreased from approximately 90 to 32, an increase was observed from 2021 onwards. The number of reported cases reached 60 in 2023, followed by a slight decline to 45 cases in 2024.

Pearson's correlation analysis between annual human surgical cases and animal cases detected during 2014–2024 revealed a weak positive association ($r = 0.29$). However, this correlation was not statistically significant ($p = 0.392$), indicating that annual fluctuations in human and animal cases were not directly correlated during the study period.

Of the 995 cases included in the sex-specific analysis, 470 (47%) occurred among males and 525 (53%) among females (Table 1). Although the distribution of cases was relatively balanced between sexes, females exhibited a slightly higher average incidence rate (2.0 per 100,000 population) compared with males (1.7 per 100,000 population).

Table 1. Distribution of cases of CE among individuals by sex, 2011-2024, n=995.

Sex	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	Total
M	46	71	64	49	33	32	17	14	32	18	17	26	29	22	470
F	51	77	85	47	34	31	28	26	25	14	19	34	31	23	525
T	97	148	149	96	67	63	45	40	57	32	36	60	60	45	995
M%	47	48	43	51	49	50	38	35	56	56	47	43	48	49	47
F%	53	52	57	49	51	49	62	65	44	43	53	57	52	51	53

Regarding anatomical localization, the liver was the most frequently affected organ, accounting for 76% of cases, followed by the lungs (17%). Less common localizations included the spleen, brain, kidneys, bones, muscles, and pelvic cavity. Multiple-organ involvement was also observed, including combinations such as liver and spleen or liver and lung. Primary forms of the disease represented 89% of cases, whereas recurrent infections accounted for 11%.

Analysis by place of residence revealed that most patients originated from rural areas (77.8%). The average incidence in rural populations was 1.8 cases per 100,000 inhabitants, compared with 0.5 cases per 100,000 inhabitants in urban areas. This pattern is consistent with the epidemiology of cystic echinococcosis, which is closely associated with livestock breeding and exposure to domestic animals involved in the parasite life cycle.In 2024, the highest incidence rates were recorded in Ceadîr-Lunga (12.6 per 100,000 population), Drochia (6.2), Taraclia (6.1), Basarabeasca and Florești (6.0 each), Leova (5.7), Ocnița (4.8), and Telenești (4.3). These values considerably exceeded the national incidence rate of 1.7 cases per 100,000 population, indicating the persistence of areas with elevated disease burden.

Surveillance and monitoring of CE in animals

The epidemiological assessment of cystic echinococcosis (CE) in animals was based on official data obtained from the databases of NAFS (Table 2).

Table 2. Number of animals slaughtered and structure of cases of CE registered in the Republic of Moldova, 2014-2023.

No. anim. Slaughtered	Cattle			Sheep			Horse			Pigs		
Year	Total No. anim.	No. cases CE	%	Total No. anim.	No. cases CE	%	Total No. anim	No. cases CE	%	Total No. anim.	No. cases CE	%
2014	32636	**2519**	7.7	32724	**4834**	14.8	551	**3**	0.5	309309	**6091**	2.0
2015	36001	**2442**	6.8	23120	**4143**	17.9	514	**12**	2.3	261815	**6237**	2.4
2016	38177	**2755**	7.2	34917	**3163**	9.1	597	**4**	0.7	292124	**4094**	1.4
2017	41890	**3026**	7.2	96035	**6539**	6.8	1210	**11**	0.9	267132	**4250**	1.6
2018	40197	**2367**	5.9	96534	**3942**	4.1	1141	**4**	0.3	344943	**3635**	1.1
2019	31155	**1439**	4.6	74463	**4595**	6.2	1512	**1**	0.1	322379	**2519**	0.8
2020	25880	**3032**	11.7	71461	**2013**	2.8	1401	**8**	0.6	380865	**3611**	0.9
2021	24282	**899**	3.7	59017	**6640**	11.2	683	**9**	1.3	378550	**1670**	0.4
2022	20103	**786**	3.9	28288	**4358**	15.4	367	**0**	0.0	391457	**1423**	0.4
2023	20719	**511**	2.5	46942	**896**	1.9	114	**4**	3.5	338118	**1413**	0.4
Total	**311040**	**19776**	**6.4**	**563501**	**41123**	**7.3**	**8090**	**56**	**0.7**	**3286692**	**34943**	**1.1**

Note: Total animals slaughtered: n=4,169,323
Total CE cases in animals: n=95,898

The analysis included post-mortem inspection records from veterinary-authorized slaughterhouses in the Republic of Moldova during the period 2014-2023. A total of 4,169,323 animals were examined during the study period. Pigs represented the largest proportion of slaughtered animals (78.8%), followed by sheep (13.5%), cattle (7.5%), and horses (0.2%). The overall number of animals slaughtered increased over time, primarily due to a growing number of pigs processed in authorized slaughterhouses.

Overall, 95,898 cases of CE were identified, corresponding to an average prevalence of 2.3% among all examined animals (Table 2). These findings indicate the continued circulation of *Echinococcus granulosus* within livestock populations in the Republic of Moldova.

The highest average prevalence was recorded in sheep (7.3%), followed by cattle (6.4%), pigs (1.1%), and horses (0.6%). Among cattle, prevalence decreased from 7.7% in 2014 to 2.5% in 2023, representing an approximately threefold reduction during the study period. A temporary increase was identified in 2020, when prevalence reached 11.7%.

Among sheep, prevalence showed greater annual variability. The highest values were recorded in 2015 (17.9%) and 2022 (15.4%), whereas a substantial decline was observed in 2023, when prevalence decreased to 1.2%.

In pigs, a consistent downward trend was observed throughout the study period. Prevalence decreased from 2.4% in 2015 to 0.4% in 2023, representing a reduction of more than fivefold. Similar decreasing trends were observed in the other livestock species, although the magnitude of change varied between species (Figure 4).

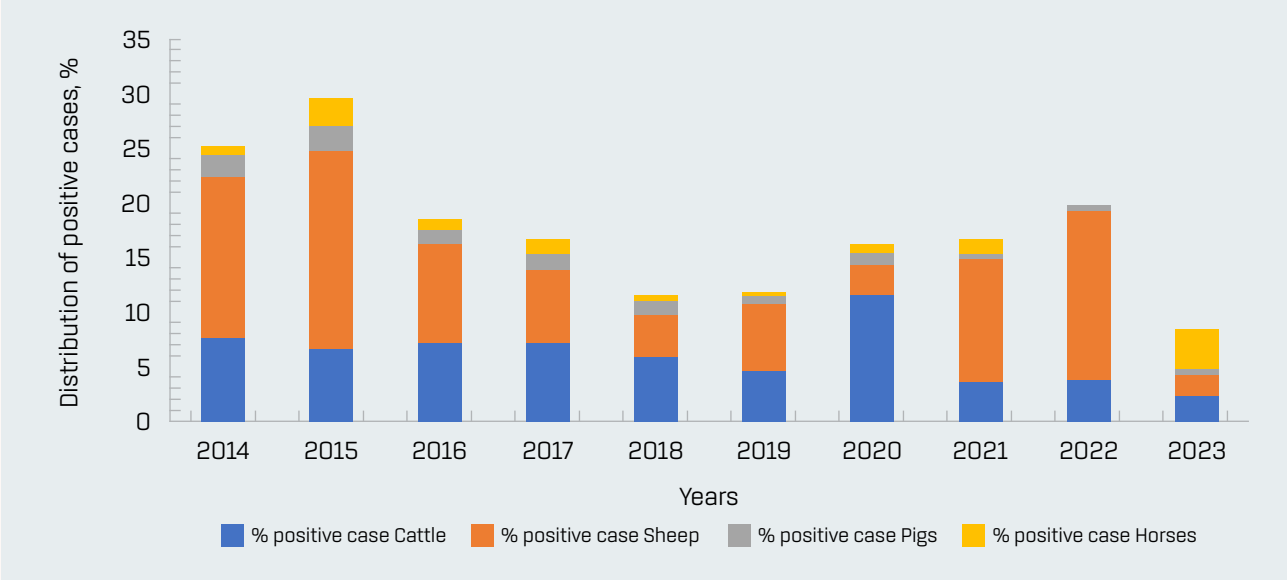

Figure 4. Distribution of echinococcosis cases %, among slaughtered farm animals, Moldova, 2014-2023

Overall, the declining prevalence observed in livestock suggests a reduction in the occurrence of cystic echinococcosis among slaughtered animals during the study period. Nevertheless, the continued detection of positive cases across all major livestock species indicates the persistence of the parasite cycle and highlights the importance of maintaining veterinary surveillance and control measures.

DISCUSSION

The present study demonstrates that CE remains endemic in the Republic of Moldova, despite a declining trend in both human cases and animal prevalence over the study period. Although the annual number of surgically confirmed human cases decreased substantially between 2011 and 2020, a resurgence was observed after 2021. Similarly, the prevalence of CE in slaughtered livestock declined markedly between 2014 and 2024, suggesting improvements in disease control at the animal level.

The incidence rates observed in Moldova remain considerably higher than those reported in the European Union. While EU/EEA notification rates ranged between 0.16 and 0.19 cases per 100,000 population in 2021–2022 (14, 15) the incidence in Moldova reached 2.9 cases per 100,000 population in 2023. These findings are consistent with reports identifying southeastern Europe as one of the remaining endemic regions for cystic echinococcosis (13).

The predominance of cases among adults, particularly those aged 31–60 years, is consistent with the natural history of CE. Due to the slow growth of hydatid cysts and the prolonged asymptomatic phase of infection, diagnosis frequently occurs years after exposure (5). The occurrence of cases among children indicates continued transmission of the parasite and ongoing exposure in some communities. The marked predominance of cases in rural areas (77.8%) and the higher incidence observed among rural populations (1.8 vs 0.5/100,000) are consistent with previous studies demonstrating the close association between CE transmission, livestock farming, and contact with domestic dogs, the definitive hosts of *Echinococcus granulosus* (5, 21, 24-26). The heterogeneous geographical distribution observed across the country further suggests the existence of local epidemiological factors that may influence transmission dynamics.

The predominance of hepatic (76%) and pulmonary (17%) involvement corresponds to the classical clinical presentation of CE reported in the literature, where the liver and lungs represent the primary filtering organs for parasite larvae following intestinal absorption (5). The proportion of recurrent cases (11%) reflects the chronic nature of the disease and the need for long-term follow-up.

The veterinary component of the study demonstrated a substantial reduction in CE detection among slaughtered animals, from 24.5% in 2014 to 4.7% in 2024, particularly in sheep and cattle, which are recognized as the main intermediate hosts in the transmission cycle. Similar slaughterhouse-based methodologies have been used to monitor CE in endemic regions (22). However, the continued detection of infected animals across all major livestock species indicates that transmission has not been completely interrupted. The discrepancy between declining animal prevalence and the persistence of human cases may be explained by the long incubation period of CE, differences in surveillance systems, and the cumulative effect of exposures acquired years before diagnosis.

The temporary increase in human incidence after 2021, despite declining livestock prevalence, may reflect reporting variability, time-lag between infection and surgery, or differences between regulated and informal livestock systems (5, 14).

The correlation analysis between annual human and animal cases revealed a weak positive but statistically non-significant association ($r = 0.29$; $p = 0.392$). This result is not unexpected considering the biological and epidemiological characteristics of CE. Human cases frequently represent infections acquired many years before diagnosis, whereas animal cases detected at slaughter reflect more recent transmission events.

Although dogs are biologically recognized as definitive hosts in CE transmission (5, 20, 21) this study did not include dog-related indicators; therefore, their specific contribution cannot be quantified from the present data. Future One Health evaluations should integrate definitive host surveillance and community-level practices to better explain territorial clustering and temporal trends (16). These findings highlight the complexity of CE transmission and underscore the importance of integrated surveillance systems encompassing both human and animal health sectors. Recent One Health frameworks advocate the integration of epidemiological, veterinary, environmental, and laboratory data to improve the detection, monitoring, and control of zoonotic diseases, including cystic echinococcosis (6-8). The limited availability of integrated human-animal surveillance data remains a challenge in many endemic countries and has been identified as an important barrier to effective CE control programs (8, 11).

STUDY LIMITATIONS

This study has several limitations that should be considered when interpreting the results:

- The use of aggregated surveillance data limited the possibility of performing individual-level risk factor analyses and more advanced multivariable statistical modelling. Therefore, the findings should be interpreted primarily as descriptive and exploratory.
- The veterinary data were derived exclusively from animals slaughtered in NAFS-authorized slaughterhouses, which may not fully represent the epidemiological situation in backyard or informal slaughter settings.
- There was a lack of detailed data regarding the anatomical localization of cysts in slaughtered animals, limiting the possibility of comparative analysis with human clinical forms.
- The absence of disaggregated data on the territorial distribution of animal cases (by districts) restricted the ability to perform a comprehensive spatial analysis and direct correlation with human incidence patterns.
- The study did not include data on definitive hosts (dogs), which play a key role in the transmission of *Echinococcus granulosus*, thus limiting the comprehensive assessment of transmission dynamics within the One Health framework.
- Potential underreporting in human cases, may have led to an underestimation of the true burden of disease.

CONCLUSIONS

Cystic echinococcosis remains an endemic zoonotic disease in the Republic of Moldova, with incidence consistently higher among adults than children, though continued cases among children and adolescents point to ongoing exposure in certain communities. The geographical distribution is heterogeneous, with several districts exceeding the national average in 2024, and most cases (77.8%) originating from rural areas, where incidence significantly surpasses urban settings, underscoring a strong association with rural living environments.

Human cystic lesions predominantly affect the liver and lungs, although complex forms involving multiple sites or vital organs, including the spleen, kidneys, brain, and bones were also documented; the occurrence of relapses

further emphasizes the need for sustained clinical follow-up and appropriate medical and surgical management. On the veterinary side, sheep showed the highest prevalence of infection, followed by cattle and pigs, although slaughterhouse data, limited to NAFS-authorized facilities, likely underrepresent informal or household-level slaughter practices.

Taken together, these findings highlight the need for strengthened intersectoral collaboration under a One Health framework, with future efforts focused on integrated surveillance, improved data exchange between human and veterinary sectors, targeted prevention in high-incidence areas, and further research into the community-level drivers of transmission.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

Ethical approval was not required, as the study used anonymized, aggregated surveillance data and did not involve direct contact with human participants or animals.

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PREGNANT WOMEN'S CRITERIA FOR SELECTING OBSTETRIC CARE PROVIDERS

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ABSTRACT

Introduction Recent health system reforms in the Republic of Moldova have given pregnant women greater choice in selecting a maternity facility for childbirth. This study aimed to examine women's views on the selection of obstetric care providers, identify key decision-making criteria, and develop recommendations for targeted marketing strategies in maternity care.

Materials and methods A quantitative cross-sectional study was conducted among 533 pregnant women recruited using non-probability convenience sampling. A study-specific structured questionnaire was administered both online and in paper format through healthcare institutions. Statistical analyses were performed using SPSS and Microsoft Excel to identify and quantify the factors influencing the selection of obstetric care providers and maternity facilities.

Results The choice of maternity facility was influenced by a combination of medical, socioeconomic, institutional, and individual factors. The criteria most frequently rated as highly important were the professional competence of medical staff (84.8%; 95% CI: 81.49–88.12), the availability of modern medical equipment (70.8%; 95% CI: 66.22–75.38), and adherence to appropriate hygiene standards in the maternity ward (64.0%; 95% CI: 58.91–69.09).

Conclusions The choice of maternity facility is a complex decision-making process, shaped by careful evaluation of prior experiences, institutional reputation, the professionalism of medical personnel, available facilities and services, and access to up-to-date information.

Keywords Pregnancy, pregnant women, maternity services, obstetric care, healthcare provider selection, maternity hospital, decision-making, patient choice.

CRITERIILE DE SELECȚIE A PRESTATORILOR DE SERVICII OBSTETRICALE DE CĂTRE FEMEILE ÎNSĂRCINATE

Introducere Reformele sistemului de sănătate din Republica Moldova au extins posibilitățile femeilor însărcinate în alegerea instituției în care doresc să nască. Scopul acestui studiu constă atât în evaluarea percepțiilor femeilor însărcinate privind selectarea furnizorilor de servicii obstetricale, identificarea principalilor factori care stau la baza deciziei, cât și formularea unor recomandări bazate pe dovezi pentru dezvoltarea unor strategii de marketing țintite în cadrul instituțiilor de asistență obstetricală.

Materiale și metode A fost realizat un studiu cantitativ transversal, care a inclus 533 de femei însărcinate, recrutate printr-o metodă de eșantionare de conveniență. Datele au fost colectate prin intermediul unui chestionar structurat, elaborat special pentru acest studiu și administrat onlineși în format tipărit în cadrul instituțiilor medicale. Analiza statistică a fost efectuată, utilizând programele SPSS și Microsoft Excel, în vederea identificării și cuantificării factorilor care influențează alegerea furnizorului de servicii obstetricale și a instituției de maternitate.

Rezultate Rezultatele studiului demonstrează că alegerea instituției de maternitate reprezintă un proces decizional multifactorial, influențat de factorii medicali, socioeconomici, instituționali și individuali. Printre criteriile apreciate de femeile însărcinate ca fiind de importanță majoră au fost: competența profesională a personalului medical (84,8%;IC 95%:81,49–88,12); disponibilitatea echipamentelor medicale moderne în cadrul instituției (70,8%;IC 95%: 66,22–75,38) și respectarea standardelor corespunzătoare de igienă în maternitate (64,0%;IC 95%: 58,91–69,09)

Concluzii Alegerea maternității constituie un proces decizional complex și multidimensional. Femeile însărcinate își fundamentează opțiunea pe o combinație de condiții, incluzând experiențele medicale anterioare, reputația instituției, competența profesională a personalului medical, disponibilitatea și calitatea infrastructurii și dotărilor medicale, precum și accesul la informații relevante, fiabile și actualizate privind serviciile de maternitate. Înțelegerea acestor determinanți poate contribui la dezvoltarea, de către instituțiile de maternitate, a unor strategii de comunicare și marketing centrate pe pacientă, adaptate mai eficient așteptărilor și prioritățiilor femeilor însărcinate.

Cuvinte-cheie Sarcină, femei însărcinate, servicii de maternitate, îngrijiri obstetricale, selectarea furnizorului de servicii medicale, maternitate, proces decizional, alegerea pacientei.

INTRODUCTION

In the Republic of Moldova, the choice of obstetric care provider has become increasingly important following recent health system reforms, growing competition between public and private maternity facilities, and the increasing use of online information, at a time of declining birth rates, migration, and a shrinking population of women of reproductive age. In most cases, the choice of a maternity hospital is not a random process, but a decision influenced by both objective and subjective factors. Pregnant women typically consider a range of criteria such as healthcare facility infrastructure, healthcare professionals' qualifications, other patients' reviews, safety, comfort, and logistical aspects including the geographical location of the maternity centre. Previous experiences and recommendations shared through online communities and social media may also influence their decisions (1). The choice of maternity facility is also influenced by women's socioeconomic status and level of education. Studies conducted in various countries show that women with higher levels of education tend to be more informed and selective, often preferring institutions that offer greater safety and comfort (2). Furthermore, research indicates that women living in urban areas are more likely to choose private maternity facilities, whereas those in rural areas tend to rely more heavily on public institutions (3, 4). The obstetrician's gender may also influence provider choice. In some conservative cultural settings, women may prefer a female physician because they perceive such care as more comfortable and reassuring. However, this preference is not universal, and for many women, the physician's experience and professional competence are more important than gender (5, 6). Overall, the criteria used to select obstetric care providers are shaped by local cultural norms and social expectations, supporting the need for a national study of these factors.

Studies emphasize that person-centered maternity care, which is responsive to women's individual preferences, needs, and values, is associated with greater patient satisfaction and may influence the choice of obstetric care provider (7). Effective communication between women and healthcare professionals is also essential to a positive childbirth experience and to building trust in the healthcare system (8).

For more than a decade, national policy in the Republic of Moldova has allowed women to choose the healthcare institution where they wish to give birth. This has broadened the range of available options, including private maternity facilities that increasingly compete with public institutions. As a result, women's preferences have become increasingly important to the sustainability and performance of obstetric care providers. The shift of pregnant women from public to private maternity facilities may have important financial implications for publicly funded institutions, whose reimbursement from the National Health Insurance Company depends on the number and complexity of births attended. Identifying the factors that influence the choice of maternity facility is therefore strategically important for both public and private healthcare institutions, which need to align their services and marketing strategies with women's expectations and needs. However, although current legislation allows women to choose the institution where they will give birth, it does not permit them to select the individual physician who will attend the delivery, which may present a challenge for women seeking continuity of care (9, 10, 11).

Aim. This study aimed to examine pregnant women's views on the choice of obstetric care providers, identify the key criteria guiding their decisions, and develop recommendations for marketing strategies tailored to maternity services.

MATERIALS AND METHODS

A descriptive, observational cross-sectional study was conducted to identify the criteria influencing pregnant women's choice of obstetric care providers. Data were collected between December 2024 and January 2025.

Study population and sample size

The target population comprised pregnant women in the Republic of Moldova. The minimum required sample size was calculated using Epi Info version 7.2.2.6 (StatCalc – Sample Size and Power), assuming a 95% confidence level, a 5% margin of error, a reference population of 24,033 births registered in the Republic of Moldova in 2023, a design effect of 2, and an anticipated non-response rate of 10%. After adjustment, the required sample size was 533 participants. However, because participants were recruited using non-probability convenience sampling through healthcare institutions and a social media group, the sample cannot be considered representative of all pregnant women in the Republic of Moldova. The findings should therefore not be generalised beyond the study population and apply primarily to pregnant women who attended antenatal care at the participating institutions and voluntarily agreed to take part during the data collection period (December 2024–January 2025).

To better reflect the distribution of births between public and private institutions, questionnaires were allocated proportionally between the two sectors: 177 to public institutions and 356 to private institutions.

Eligibility criteria

Eligible participants were pregnant women aged 18–49 years, at any gestational age, with an uncomplicated pregnancy who provided consent to participate. Women were excluded if they were younger than 18 or older than 49 years, had a complicated pregnancy, or refused to participate.

Data collection and study instrument

Data were collected using a structured questionnaire developed specifically for this study. The questionnaire comprised two sections:

(A) sociodemographic and background characteristics, including age, place of residence, education, standard of living, parity, gestational age, and place of previous delivery; and

(B) preferences regarding place of delivery and factors influencing the choice of obstetric care provider.

The questionnaire was pretested with seven pregnant women, and minor revisions were made following the pilot phase to improve clarity and comprehensibility. Items were worded to be accessible to respondents with different educational backgrounds and to minimize ambiguity and avoid leading questions. Before data collection, content validity was assessed through expert review. Specialists in public health and obstetrics evaluated the relevance, clarity, and completeness of the questionnaire items, and their feedback was incorporated into the final version. The questionnaire did not undergo full psychometric validation; accordingly, its use was limited to descriptive and association analyses within the study population.

The questionnaire was available in both paper and online formats. Paper questionnaires were distributed with the assistance of family physicians through public and private healthcare institutions providing antenatal care. Participating public institutions included the Mother and Child Centre, the "Gheorghe Paladi" Municipal Clinical Hospital, Municipal Maternity Hospital No. 2, and

family medicine centres in Comrat, Straseni, Ungheni, Cauşeni, and Anenii Noi. Participating private institutions included Repromed and Excellence. The online questionnaire was also shared through the Facebook group "Ask a Mom" to broaden recruitment among pregnant women.

Only valid responses were retained, yielding a final sample of 533 participants. Because both paper-based and online questionnaires were used, several measures were taken to reduce the risk of duplicate participation: (1) respondents were explicitly instructed at the beginning of both versions to complete the questionnaire only once; (2) the online questionnaire was configured to allow a single submission per device; and (3) during the data-cleaning phase, questionnaires were screened for identical or near-identical response patterns across key sociodemographic variables, and suspected duplicates were excluded from the final dataset. Despite these measures, the possibility of residual duplication cannot be entirely excluded and is acknowledged as a methodological limitation of this study.

Statistical analysis

The collected data were coded, tabulated, and entered into a study database. Statistical processing was performed using SPSS (Statistical Package for the Social Sciences) version 26.0 and Microsoft Excel. Descriptive statistics were used to summarize the characteristics of the study population, including frequencies, percentages, means, and standard deviations. Associations between categorical variables (e.g., preferred place of birth and sociodemographic characteristics) were assessed using the chi-square test (χ^2), with Cramér's V calculated as a measure of effect size where appropriate. For ordinal variables or in cases where the assumptions of normality were not met, Spearman's rank correlation coefficient (ρ) was applied to examine associations. The Pearson correlation coefficient was not used, as the study variables did not fulfil the prerequisites for its valid application (continuous scale and bivariate normality). All statistical tests were two-sided, and a p value <0.05 was considered statistically significant.

Participation was voluntary. All participants were informed about the purpose of the study, and confidentiality was maintained throughout data collection and analysis. No personally identifying information was collected.

RESULTS

According to the results of the study, the mean age of the participants was 27.5 ± 1.63 years. Women aged 26–33 years constituted the largest age group (234; 43.90%), followed by those aged 34–41 years (26.64%), 18–25 years (26.45%), and 42–49 years (3.00%). The distribution of participants by place of residence differed significantly between urban and rural areas ($\chi^2 = 35.64$, df = 1, $p < 0.0001$).

Financial circumstances may also affect the choice of obstetric care provider by limiting the range of options available to women. This is particularly relevant when considering private maternity care, as perinatal services provided by public healthcare institutions in the Republic of Moldova are covered by mandatory health insurance. In the present study, 35.46% of respondents (95% CI: 28.77–42.29) reported having sufficient financial resources for a decent standard of living but not for more expensive purchases, while 29.46% (95% CI: 22.33–36.59) stated that their income covered only basic needs. A further 21.76% (95% CI: 14.25–29.27) reported being able to afford some more expensive items, although this required reducing expenditure in other areas. Only 6.19% (95% CI: –2.03–14.41) reported having no financial constraints, whereas

7.13% (95% CI: 5.24–9.63) stated that their income was insufficient even to cover basic necessities. Overall, these findings suggest that women's standard of living may influence their choice of obstetric care provider and contribute to differences in preferences for public and private maternity facilities.

The study showed that pregnant women with higher education predominated in the sample, accounting for 301 respondents (56.5%), followed by those with secondary education (140; 26.3%), lower secondary education (71; 13.3%), and primary education (21; 3.9%). These findings indicate that the study population was composed predominantly of women with a higher educational background, a factor that may influence awareness of available obstetric care options and expectations regarding the quality of services. Most pregnant women included in the study were employed, comprising 308 respondents (57.79% of cases; 95% CI: 51.34–64.24).

Most participants were married or in a relationship (505; 94.75%), while 28 (5.25%) were single; this difference was statistically significant ($\chi^2 = 236.69$, df = 1, $p < 0.0001$). Approximately two-thirds of the women were expecting their second or subsequent child, whereas one-third had no children before the current pregnancy. At the time of the survey, 40.9% of participants were at 22–37 weeks' gestation, 38.84% were at less than 20 weeks, and 20.26% were beyond 37 weeks. These findings suggest that women in the Republic of Moldova make decisions about their maternity facility at different stages of pregnancy.

At the time of data collection, most women had already decided to give birth in a public maternity facility (61.73%; 95% CI: 54.90–68.56). The findings also suggest a shift in the anticipated mode of delivery, with a higher proportion of participants planning a caesarean section than had undergone one in previous pregnancies. Vaginal birth remained the most commonly planned mode of delivery (58.91%), while 21.76% of women reported a planned caesarean section based on clinical indications and/or an individualized obstetric management plan. For the remaining 19.32%, the mode of delivery had not yet been determined at the time of questionnaire completion.

Regarding the preferred gender of the obstetrician, 53.28% of participants preferred a female obstetrician, 21.76% preferred a male obstetrician, and 24.95% had no gender preference. These findings reflect participants' personal preferences and should not be interpreted as implying a general right to choose an individual healthcare provider within public healthcare institutions. The distribution of preferences was statistically significant ($\chi^2 = 10.22$, df = 2, $p = 0.0217$).

Further analysis showed that 43.71% of women (95% CI: 37.34–51.24) chose their maternity facility because their preferred physician was affiliated with that institution. In 13.70% of cases (95% CI: 5.48–21.92), the decision was based on recommendations from acquaintances, while 16.32% (95% CI: 8.14–24.52) chose the maternity hospital assigned to their area of residence. Only 2.25% (95% CI: 1.29–3.89) selected a facility because they considered it the most prestigious in the region. In addition, 13.51% (95% CI: 5.29–18.73) based their choice on a previous positive childbirth experience at the same institution. Finally, 10.51% of respondents (95% CI: 3.34–16.52) reported that the particular maternity facility was not important to them. Overall, the findings indicate that approximately half of the women preferred a maternity facility where the physician they wanted to attend their delivery was based. This is consistent with previous studies showing that continuity of care and a trusting relationship with the physician who provided antenatal care are important factors in women's choice of place of birth (6).

The person most frequently reported as influencing the choice of obstetric care provider was a close relative (23.26%; 95% CI: 15.82–30.61), followed by the gynecologist (18.39%; 95% CI: 10.72–25.96) and friends or neighbors (11.82%; 95% CI: 3.85–19.79). These findings suggest that pregnant women take an active role in selecting their obstetric care provider and place considerable trust in their own judgement, particularly when supported by information from official institutional websites and parenting groups on social media. At the same time, recommendations from trusted individuals, especially their gynecologist or a close relative, remain an important influence.

For most participants, the choice of obstetric care provider was based on both the healthcare institution and the attending physician (54.97%; 95% CI: 49.27–60.66), followed by the physician alone (35.65%; 95% CI: 28.84–42.46). By contrast, the institution alone was much less frequently considered decisive (7.13%; 95% CI: –1.05–15.31). Thus, for approximately half of the pregnant women in the study, both the healthcare institution and the obstetricians practising there were important when deciding where to give birth. For about one in three women, however, the obstetrician alone was the determining factor in the choice of obstetric care provider.

To examine the factors underlying pregnant women's choice of obstetric care providers, the analysis was structured around the four traditional components of the marketing mix: product, price, place, and promotion. Among the factors assessed, those related to the "product" component, defined here as the services offered to meet patients' needs, were rated as the most important. In particular, the professional competence of physicians was considered important by 84.8% of respondents (95% CI: 81.49–88.12), the availability of medical equipment by 70.8% (95% CI: 66.22–75.38), and perceived compliance with hygiene standards in the maternity ward by 64.0% (95% CI: 58.91–69.09). These results reflect participants' perceptions and self-reported selection criteria rather than objective measures of healthcare quality. Such perceptions may have been influenced by personal experience, recommendations from healthcare professionals, relatives or acquaintances, online information, antenatal education activities, and other available sources of information.

Within the promotion component, the factors rated as most important when choosing an obstetric care provider were respectful staff attitudes (58.9%; 95% CI: 54.72–63.08), clear and understandable communication (58.1%; 95% CI: 53.91–62.29), and the physician's willingness to provide patient and thorough answers (56.9%; 95% CI: 52.70–61.10). According to the study results, pregnant women considered several price-related criteria, as a component of the marketing mix, to be important when selecting an obstetric service provider. These included the integrity of medical personnel, defined as not requesting or accepting informal payments, cited by 45.4% of respondents (95% CI: 41.17–49.63), coverage of services by mandatory health insurance, mentioned by 31.0% (95% CI: 27.07–34.93), and the actual cost of services, highlighted by 29.1% of participants (95% CI: 25.24–32.96). The latter criterion is primarily relevant to private healthcare institutions, as perinatal care provided within public healthcare institutions in the Republic of Moldova is fully covered by the mandatory health insurance system.

Another key component of the marketing mix, place, was also considered important. The main criteria were good postpartum hospital conditions, reported by 48.8% of respondents (95% CI: 44.56–53.04), and proximity to the maternity facility, cited by 18.4% (95% CI: 15.11–21.69).

Among the information channels influencing the choice of place of birth, the most frequently rated as very important were official institutional websites providing information on services and physicians (39.77%; 95% CI: 33.18–

46.36), educational sessions for expectant mothers organized by medical institutions (23.31%; 95% CI: 16.19–30.43), and the institutions' social media pages (22.89%; 95% CI: 15.74–30.04). In addition, the availability of key specialists and critical care services was considered particularly important when selecting an obstetric care provider: obstetrician-gynecologists were rated as very important by 83.11% of respondents (95% CI: 78.02–88.22), neonatologists by 79.70% (95% CI: 73.71–85.69), and intensive care and resuscitation services for newborns and adults by 72.61% (95% CI: 66.49–78.73).

DISCUSSION

The age distribution in our sample is consistent with official statistics showing that most births in the Republic of Moldova occur among women aged 25–34 years. This pattern is also consistent with published evidence suggesting that maternal age may be associated with the choice of obstetric care provider. Younger women tend to choose public maternity facilities, partly because of their greater accessibility, whereas women aged 34–49 years may be more likely to opt for private facilities offering more personalized care and greater comfort (1, 2).

Place of residence was also significantly associated with the choice of obstetric care provider in our study. Women living in urban areas generally have better access to large, well-equipped hospitals and may be more likely to choose private facilities because of the wider availability of personalized services. In contrast, women living in rural areas may have more limited access to well-resourced healthcare facilities and may therefore rely on regional public hospitals or travel to urban centers for more advanced care (4).

Higher educational attainment appears to be associated with a more informed and deliberate choice of obstetric care provider, as well as higher expectations regarding hospital conditions, physicians' attitudes, and available childbirth options. In contrast, women with lower levels of education may be more likely to use public maternity services, potentially reflecting differences in access to care, financial resources, and patterns of healthcare use (8).

Occupation may also influence the choice of maternity facility through its relationship with income, working hours, educational attainment, and working conditions. Women in higher-paid occupations may be more likely to choose private maternity facilities offering a broader range of services. In addition, flexible working arrangements may make it easier to attend antenatal appointments and may influence preferences for facilities that offer appointment times compatible with women's schedules (9).

Furthermore, women with higher educational attainment, which may be associated with certain occupational groups, tend to have greater access to information about available maternity care options and may therefore be more likely to choose facilities with a strong reputation or access to specialized services (10, 11).

Marital status may also influence the choice of maternity facility, as it can affect financial resources, emotional and partner support, social circumstances, and access to healthcare-related or legal benefits. Married women may have greater financial and partner support, potentially broadening their options, including access to private maternity services. In contrast, unmarried women may be more likely to rely on public services or prefer facilities that offer greater psychological support, confidentiality, and sensitivity to their individual circumstances (11).

According to several sources, parity may also influence the choice of maternity facility, depending on previous childbirth experiences, individual needs, and

available resources (1, 2, 10). Parents who have had a positive experience at a particular maternity hospital with their first child may be more likely to return to the same institution for subsequent births. Conversely, a negative experience related to the quality of care or hospital conditions may lead them to seek an alternative facility. In addition, families with several children may face greater household expenses and therefore consider more affordable options, such as public maternity hospitals rather than private facilities. On the other hand, unsatisfactory previous experience in a public maternity facility may lead some families to allocate a larger budget for private maternity care in a subsequent pregnancy (9). The literature also indicates that previous mode of birth and obstetric history may influence the choice of maternity facility for subsequent deliveries (1). For example, women with a previous uncomplicated vaginal birth may prefer a less medicalized birth setting for their next delivery. In contrast, women with a history of complicated vaginal birth or caesarean section may prioritize facilities with expertise in caesarean delivery, experience in managing vaginal birth after caesarean, and access to appropriately qualified specialists.

Our findings are consistent with previous studies suggesting that the obstetrician's gender may influence the choice of maternity facility, depending on women's personal preferences, cultural background, and previous experiences (6). However, the literature also indicates that the quality of care does not differ significantly between male and female obstetricians (6).

The availability of specialist clinicians and appropriate clinical units within a maternity facility is another important consideration when choosing where to give birth, as it may provide greater reassurance and ensure comprehensive care for both the mother and newborn (10).

When compared with findings from the literature, the results of the present study are consistent in showing that pregnant women tend to prioritize a specific medical team as well as access to medical technologies available within a healthcare institution (7, 8). In addition, the obstetrician-gynecologist has an important influence on the choice of obstetric care provider through clinical expertise, professional recommendations, and the trusting relationship established during pregnancy (6).

Promotional activities may also influence women's choice of obstetric care provider, particularly in a competitive healthcare environment where the decision to give birth at a particular maternity hospital is shaped not only by clinical competence but also by women's perceptions of, and trust in, the institution (3, 4).

LIMITATIONS

Several limitations should be considered when interpreting the findings. First, the use of convenience sampling limits the external validity of the study, and the results should not be generalized to all pregnant women in the Republic of Moldova. Second, the use of both paper-based and online questionnaires introduced a risk of duplicate participation; although data-cleaning procedures were used to minimize this risk, it could not be eliminated entirely. Third, the cross-sectional design does not allow causal relationships to be established. Fourth, the predominance of women with higher educational attainment and urban residence may limit the extent to which the sample reflects the wider population of pregnant women, particularly those living in rural areas or experiencing socioeconomic disadvantage. Finally, the questionnaire did not undergo full psychometric validation, which may limit the precision with which subjective constructs, such as perceived quality of care, were measured.

CONCLUSIONS

The study demonstrates that all four components of the marketing mix—product, price, promotion, and place—influence pregnant women's choice of obstetric care provider. The most important factors were related to the product component, particularly the professionalism of medical staff, medical equipment and facilities, and hygiene standards, followed by promotion (respectful attitudes and clear communication), price (transparent fees and the absence of informal payments), and place (proximity and postpartum inpatient conditions). As technology and access to information continue to evolve, healthcare institutions need to strengthen their communication practices and adapt their services to women's needs and preferences. An integrated approach, supported by sustained investment, can help maternity facilities remain responsive to patient expectations while providing safe, high-quality care.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ETHICAL ISSUES

This study was conducted in accordance with the ethical standards of the Declaration of Helsinki and was approved by the relevant institutional ethics committee. All participants were informed about the purpose of the research and gave their voluntary, written informed consent prior to participation. Anonymity and confidentiality were ensured throughout the study, and no personal identifying information was collected or disclosed. Participants were also informed of their right to withdraw from the study at any time without consequence. The research involved no physical or psychological risk to the participants. The study did not receive any funding that could lead to a potential conflict of interest.

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REQUIREMENTS FOR AUTHORS

Rules of drafting

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; original articles – 3,000 words; opinions/perspectives – 2,500 words; case reports / clinical-laboratory images – 1,700 words; short experimental/clinical notes – 1,300 words; book reviews – 2,000 words; didactic (educational) articles – 4,000 words. The volume of tables and figures should not exceed 1/3 from the volume of the manuscript. The journal reserves the right to make any other formatting changes.

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Title and authors

The title must be as concise as possible (maximum 120 characters including spaces) and clearly reflect the content of the manuscript. The names of the authors should be written in full: name, surname (e.g.: Ion RUSU). Affiliation should include: Department/Unit/Chair, University/Hospital, City, Country for each author. The corresponding author must provide a valid e-mail address, preferably an institutional one (e.g., Corresponding author: Ion RUSU, email: ion.rusu@usmf.md).

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The manuscript should comprise the following sub- headings (capitalized):

- **SUMMARY** (Structured Abstract)
- **INTRODUCTION**
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- **RESULTS**
- **DISCUSSION**
- **CONCLUSIONS**
- **DECLARATIONS** (Conflict of Interest, Acknowledgements and Funding, Ethics, Contributions)
- **REFERENCES**

Case Report

Recommended sections:

- **ABSTRACT**
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- **CASE PRESENTATION**
- **DISCUSSION**
- **CONCLUSIONS**
- **DECLARATIONS** (Conflict of Interest, Acknowledgements and Funding, Ethics)
- **REFERENCES**

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

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Text included in figures must be written in Cambria font, size 10 pt. Each figure must be accompanied by a title and legend. Figures must be numbered with Arabic numerals and cited in the text in round brackets (e.g., Figure 1). The figure title (e.g., Figure 1) and legend must be centered below the figure.

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The *One Health* concept

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.

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 ENVIROMENTAL 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.

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