

Review

of the dissertation for the award of the educational and scientific degree "Doctor"

by Chiydem Baarieva Ismailova,

Field of Higher Education: 4. Natural Sciences, Mathematics, and Informatics;

Professional Field: 4.3. Biological Sciences;

Scientific Specialty: Virology

Dissertation titled: **“Studies on the Serological and Molecular-Genetic
Characteristics of Hepatitis E Virus”**

Scientific Supervisor: Assoc. Prof. Lyubomira Nikolaeva-Glomb, MD, PhD

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Jury appointed by Order No. 182 / 30.06.2026 of the Director of the NCIPD

1. Biography

Chiydem Baarieva Ismailova was born in the city of Ruse. In 2013, she graduated from the "Apostol Arnaudov" Vocational High School of Electrical Engineering and Electronics, specializing in Computer Engineering and Technologies.

In 2017, she graduated from Sofia University "St. Kliment Ohridski" with a Bachelor's degree in Molecular Biology, and in 2019, she obtained a Master's degree in Microbiology and Microbiological Control from the same university.

Since then and to the present day, she has been working as a biologist at the National Center of Infectious and Parasitic Diseases (NCIPD) in Sofia—specifically, at the National Reference Laboratory for Hepatitis Viruses.

Biologist Ismailova's professional interests lie in the field of viral hepatitis diagnostics; in this regard, she continues her postgraduate professional development by participating in various forms of training:

- Annual meeting on Hepatitis B and C surveillance, organized by the European Centre for Disease Prevention and Control (ECDC, Sweden),
- Training on sequencing and phylogenetic analysis in Luxembourg,
- Completed courses in: a) Laboratory diagnosis of viral infections – NCIPD, b) Immunology and allergology,
- Application of next-generation sequencing in clinical microbiology and infectious disease diagnostics, organized by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID).

Her interests focus on the serological and molecular-biological diagnosis of hepatitis E, and she has been working on this topic for several years.

2. Relevance of the problem

Hepatitis E virus (*HEV*) was discovered approximately 40 years ago, marking the beginning of research into the virus itself and the diseases it causes. According to WHO data, hepatitis E is the most common enterically transmitted form of hepatitis, yet only a small proportion of infected individuals develop clinical symptoms. Various

genotypes of the virus have been identified, differing in their geographic distribution and the clinical course of the disease—an aspect in which *HEV* is unique.

Research into the disease has characterized its clinical manifestations and complications, and has identified extrahepatic manifestations associated with the virus. Data have also been gathered regarding the risk groups for infection and for a severe course of Hepatitis E. Of particular interest is the fact that the genotype primarily circulating in Europe has an animal reservoir, and research in this area is continuously expanding. The clinical manifestations of acute hepatitis E do not differ from those of other forms of hepatitis, and laboratory diagnostics (serological and/or molecular-biological) are crucial for establishing an etiological diagnosis. Up to 2010, there were only isolated reports from Bulgaria, but since then, the number of publications on the subject has grown; to date, one dissertation has been successfully defended, two more are in progress, and two monographs have been published.

HEV infection is of significant importance to our country as well; for this reason, in 2019, the disease—"Acute Hepatitis E"—was included in "Ordinance No. 21 on the Procedure for Registration, Notification, and Reporting of Communicable Diseases."

Over the disease's 40-year history, various diagnostic tests have been developed; however, shortcomings have been identified, leading to continuous refinement of the tests over the years. This necessitates collaboration among clinicians, biologists, and virologists, as well as careful interpretation of results to establish an accurate diagnosis.

The topic of Chiydem Baarieva Ismailova's dissertation—"Studies on the Serological and Molecular-Genetic Characteristics of the Hepatitis E Virus"—is highly relevant, and the work itself is timely; it will provide greater insight into the prevalence of this

infection among the Bulgarian population and the genotypes and sub-genotypes circulating in the country, thereby contributing to a more precise interpretation of patient test results and the development of prevention and control practices for this relatively new infection.

3. Understanding the problem

Chiydem Baarieva Ismailova is well-versed in current advances regarding Hepatitis E virus infection; the literature review in her dissertation encompasses 195 sources reflecting global knowledge on the subject—spanning the period from the virus's discovery to the present day. Eleven /11/ of these sources are in Bulgarian. The candidate herself is the lead author or co-author of five /5/ publications on Hepatitis E, has delivered presentations on the topic at twelve /12/ Bulgarian and international forums, and has participated in three /3/ scientific projects related to the subject.

4. Overview of the dissertation

The dissertation is structured in accordance with the requirements of the Regulations for the Implementation of the Development of Academic Staff in the Republic of Bulgaria Act and comprises: a title page, table of contents, list of frequently used abbreviations, introduction, literature review, aim and objectives, materials and methods, results, discussion, conclusions, contributions, references, and appendices. It contains 24 tables and 30 figures. The appendices include one additional table and two figures providing further clarification.

Introduction – 1 page – outlines the current global and national (Bulgarian) relevance of the topic.

Literature Review – 24 pages – *includes brief historical background and the current classification of *HEV* strains; *provides a detailed description of the *HEV* structure, proteins, and genome, as well as the various genotypes and sub-genotypes; *presents a detailed overview of the infection's epidemiology—including transmission mechanisms, sources of infection, geographical distribution of the different viral genotypes, and risk groups for infection and severe disease progression. Although Ismailova is not a physician, the pathogenesis, pathological findings, clinical manifestations, and the most common extrahepatic manifestations as well as modern treatment, are well presented.

Diagnostic methods for this infection are detailed, outlining the advantages and disadvantages of each method and specifying the patient groups for which particular methods are recommended. The literature review concludes with a section on infection prevention and control, outlining various measures for both endemic and non-endemic regions and presenting data on currently available vaccines.

The aim and objectives – 1 page – clearly and concisely formulated. There are four objectives, all of which are addressed in the dissertation.

Materials and methods – 18 pages: 2,467 samples (serum or whole blood) collected from across the country were analyzed — 1,863 prospective and 644 retrospective. The prospective samples analyzed were divided into two major groups — individuals with liver diseases and those with other conditions— with distinct subgroups established within each group based on the primary diagnosis. The retrospective samples were categorized into subgroups according to risk factors (e.g., immunosuppression, high-risk occupations). This classification enables a more precise interpretation of the results and the identification of risk factors and at-risk populations.

The analytical methods employed, including the statistical techniques, are described in great detail.

Results – 25 pages – the study results for the two major groups (prospective and retrospective) are presented sequentially, with the compared parameters including gender, age, and comorbidities, among others.

A study conducted on individuals in high-risk occupations and blood donors has yielded results indicating the widespread prevalence of *HEV* infection in the country, raising important questions regarding the potential for transmission through blood product transfusions and occupational exposure. Donor blood in Bulgaria is not yet screened for *HEV* markers, thereby placing recipients at risk.

A key merit of this study is the established correlation between *anti-HEV IgM* levels and the frequency of detecting viral nucleic acid in the blood samples analyzed. This is of significant practical importance: due to the short duration of viremia, *HEV RNA* is infrequently detected in samples, yet its absence does not rule out a diagnosis of acute hepatitis. In such instances, antibody levels are crucial, providing information regarding the duration of the infection. On the other hand, according to the results of studies in which Ismailova also participated, *anti-HEV IgM* antibodies can be detected in some patients up to 24 months after acute infection, and in such cases, their levels are important for the correct interpretation of the results. Data obtained from the studies conducted are analogous to those from other European countries: the *HEV3* genotype—specifically its European variants e, f, and c—circulates in the country. In other words, the viruses circulating in Bulgaria are the same as those found elsewhere in Europe, and identifying the subgenotypes is of practical importance, as some are associated with more severe disease progression.

Another important result is the identification of viruses that currently cannot be classified into known genotypes. I believe that research in this area should continue. *HEV* is a pathogen with a not fully established potential, and studies must go on.

Discussion – 11 pages: the discussion is separate from the results, which I consider appropriate; the commentary follows the order in which the results are presented, and additional citations are included to support—or otherwise—the findings obtained.

Conclusions – 1 page: the conclusions address the set objectives and are well-formulated. Contributions – 1 page: the contributions are concise, clearly formulated, and serve as a basis for further work.

Recommendations: I recommend that the group of children with onco-haematological diseases be analyzed separately, as they exhibit the highest relative prevalence of *anti-HEV IgM*. This is necessary, on the one hand, to allow for comparison with healthy individuals from the pediatric population and, on the other, because 33% of cases show cross-reactivity with *EBV* and *CMV*. The children's age suggests a likelihood of *EBV* or *CMV* infection; furthermore, their underlying onco-haematological disease points to the potential for infection or reactivation of the Herpesviruses, as well as *HEV* infection. No testing was performed for active *EBV* or *CMV* infection, nor was the study group screened for *anti-HEV IgG* antibodies. This makes interpreting the obtained results difficult.

5. Abstract – The abstract submitted by the doctoral candidate comprises 62 pages of scientific text and clearly presents the aim, objectives, results, conclusions, and contributions of the developed work.

6. Conclusion:

Hepatitis E is a disease with various clinical presentations: an asymptomatic form, acute icteric hepatitis, and chronic hepatitis in immunosuppressed individuals. In her work, the doctoral candidate describes these forms as well as the disease's diagnosis — covering serological and molecular-biological tests and specifying which methods should be applied to different patient groups to ensure an accurate diagnosis. The significance of this infection, its widespread prevalence in the country, and the challenges associated with its diagnosis are well presented. The work is timely, significant, and thorough, and it complies with the requirements of the ADASRB, its implementing regulations, and the implementing regulations of NCIPD.

Based on all of the above, I give a **positive assessment** of the dissertation by Chiydem Baarieva Ismailova and recommend that the scientific jury award her the educational and scientific degree of "Doctor" in Higher Education Area 4 (Natural Sciences, Mathematics, and Informatics), Professional Field 4.3 (Biological Sciences), and Scientific Specialty - (Virology).

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Member of the Scientific Jury appointed

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