ACADEMIC EVALUATION

On the Dissertation Submitted by
CHIYDEM BAARIEVA ISMAILOVA

entitled "STUDIES ON THE SEROLOGICAL AND MOLECULAR GENETIC CHARACTERISTICS OF HEPATITIS E VIRUS**"**

submitted in partial fulfilment of the requirements for the award of the educational and scientific degree Doctor in the scientific specialty Virology, Professional Field 4.3 Biological Sciences, Field of Higher Education 4. Natural Sciences, Mathematics, and Informatics

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

Author of the Standpoint:
Prof. Neli Stoyanova Korsun, MD, DSc
Member of the Scientific Jury appointed by Order No. 182 / 30.06.2026 of the Director of the National Centre of Infectious and Parasitic Diseases (NCIPD)

Biography

Chiydem Baarieva Ismailova graduated from the Faculty of Biology at Sofia University "St. Kliment Ohridski", where she obtained a Bachelor's degree in Molecular Biology in 2017 and a Master's degree in Microbiology and Micrological Control in 2019. From December 2018 to June 2020, she worked at the Ramus Laboratory in Sofia. She subsequently joined the National Reference Laboratory of Hepatitis Viruses at the National Centre of Infectious and Parasitic Diseases (NCIPD), where she has been employed as a biologist to the present day. From October 2020 to August 2023, she was enrolled as a full-time doctoral student at the same laboratory under the supervision of Assoc. Prof. Lyubomira Nikolaeva-Glomb. Upon successful completion of her doctoral training, she was granted the right to defend her dissertation on 31 August 2023. In March 2023, she completed a one-week training course in sequencing and phylogenetic analysis at the Luxembourg Institute of Health.

Relevance of the Dissertation Topic

Hepatitis E virus (HEV) is one of the five hepatotropic viruses and remains considerably less well studied than the other major causes of acute and chronic viral hepatitis. In Bulgaria, there is still a lack of systematic serological and molecular epidemiological studies investigating the prevalence of HEV infection, the population groups at risk, and the diagnostic approaches required to limit its spread. Consequently, expanding current knowledge of the epidemiology of HEV infection in

Bulgaria and developing effective strategies for its prevention and control underscore the relevance and scientific significance of the present dissertation.

General Characteristics of the Dissertation

The dissertation has been prepared in accordance with the requirements of the Regulations for the Implementation of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADASRB). The dissertation comprises 115 pages and is organized into the following sections: Title page; Acknowledgements (1 page); Table of Contents (2 pages); List of Abbreviations (1 page); Introduction (1 page); Literature Review (24 pages); Aim and Objectives (1 page); Materials and Methods (18 pages); Results (25 pages); Discussion (10 pages); Conclusions (1 page); Scientific Contributions (2 pages); References (19 pages, comprising 195 bibliographic references); Publications Related to the Dissertation (1 page); Participation in National and International Scientific Meetings (2 pages); Research Projects Related to the Dissertation (1 page); and Appendices (4 pages).
The proportion and organization of the individual sections comply with the accepted requirements for doctoral dissertations. The dissertation is illustrated with 24 tables and 30 figures.

Review and Evaluation of the Dissertation

In the Introduction, the doctoral candidate outlines the history of the discovery of the hepatitis E virus (HEV) and presents the various clinical manifestations of HEV infection. She emphasizes the importance of the virus's genetic characteristics in determining the clinical course and severity of the disease. She provides a well-founded rationale for investigating both the sero-prevalence of HEV in different population groups and the genetic diversity of circulating viral strains.
The Literature Review is comprehensive, well-structured, and based on up-to-date scientific evidence. It provides a thorough overview of the taxonomy, structure, and biological characteristics of HEV. The viral genome, viral proteins and their functions, and the stages of the viral replication cycle are described in considerable detail. The review also addresses the epidemiology of HEV infection, including transmission routes, geographical distribution, and risk groups, together with its pathogenesis, clinical manifestations, laboratory diagnosis, treatment, and prevention. Current serological and molecular diagnostic methods are critically presented. The chapter concludes with an overview of the epidemiological situation and the current state of HEV research in Bulgaria.
The Literature Review demonstrates the doctoral candidate's excellent command of the scientific literature and her ability to critically analyze, synthesize, and interpret current knowledge in the field.
The original research is introduced in Chapter 2: ***Aim and Objectives***. The aim of the dissertation is clearly stated—to investigate the sero-prevalence of hepatitis E virus infection and the molecular genetic characteristics of HEV isolates collected during the period 2015–2023 from a heterogeneous study population. Five specific research objectives are clearly and logically formulated, comprehensively covering the principal directions of the investigation.

The ***Materials and Methods*** employed to achieve the study aim and objectives are described clearly and comprehensively. The research included a prospective analysis of 1,823 serum samples collected from hospitalized patients with hepatic and extrahepatic diseases between 2015 and 2023. In addition, a retrospective analysis was performed on 644 serum samples obtained during the same period from a more heterogeneous study population, including veterinarians, blood donors, and other population groups. All serum samples were tested for anti-HEV IgM and IgG antibodies. The molecular investigations included the detection of HEV RNA by nested RT-PCR, quantification of viral RNA using real-time RT-PCR, and sequencing, followed by phylogenetic analysis of the detected HEV strains.

The applied methods are described accurately and in sufficient detail, demonstrating the doctoral candidate's thorough understanding of the experimental techniques and their appropriate application throughout the study.

The ***Results*** section presents the findings in three principal research directions. The first part focuses on the serological investigation, including the detection of anti-HEV IgM and IgG antibodies, followed by an analysis of the results according to the patients' sex, age, clinical diagnosis, and membership in specific risk groups. All comparisons are supported by appropriate statistical analyses, which strengthen the reliability of the reported findings.

Serum samples positive for anti-HEV IgM antibodies were subsequently analyzed by nested RT-PCR, and HEV RNA was detected in 33 samples. Ch. Ismailova further quantified the viral RNA concentration and identified a significant correlation between viral load and anti-HEV IgM antibody levels.

One of the most valuable achievements of the dissertation is the sequencing of HEV-positive samples and phylogenetic analysis, which enabled the genotyping of the detected viral isolates. All 12 successfully sequenced samples were assigned to genotype 3, representing five different subtypes (HEV-3e, HEV-3f, HEV-3c, HEV-3m, and HEV-3a). These findings demonstrate considerable genetic diversity among HEV strains circulating in Bulgaria.

The ***Discussion*** section provides a comprehensive interpretation of the obtained results and places them in the context of current scientific knowledge. The doctoral candidate critically evaluates the influence of **s**ex, age, occupation, and other risk factors on the epidemiology of HEV infection. She analyses the prevalence of HEV infection among patients with different clinical diagnoses as well as among individuals belonging to recognized risk groups. Throughout the discussion, her findings are systematically compared with those reported in studies conducted abroad.

The discussion demonstrates the candidate's ability to interpret her findings critically, relate them to the existing scientific literature, and draw well-founded conclusions regarding the epidemiology and genetic characteristics of HEV infection in Bulgaria.

In the ***concluding*** section, the doctoral candidate outlines the principal directions for future research on HEV infection, including expanding current knowledge of the clinical manifestations, clarifying its routes of transmission and risk groups, and further improving diagnostic methods. She emphasizes the significance of the present study for the development of effective strategies for the prevention and control of HEV infection in Bulgaria.

The dissertation concludes with eight clearly formulated ***conclusions***, all of which correspond directly to the stated objectives and accurately reflect the results of the conducted investigations.
I acknowledge and fully accept the four scientific ***contributions*** presented by the doctoral candidate.
The ***reference list*** comprises 195 bibliographic references, including 11 publications by Bulgarian authors, while the remaining references are international publications in English. The doctoral candidate demonstrates an excellent knowledge of Bulgarian research in this field and has appropriately incorporated these studies into the dissertation. Notably, 112 references (58%) have been published within the last ten years (2015–2025), demonstrating that the dissertation is based on current and relevant scientific literature.

Evaluation of the Abstract

The abstract comprises 69 pages and provides a concise, well-structured, and systematic presentation of the principal objectives, methodology, results, conclusions, and scientific contributions.

Publication Activity

In relation to the topic of the dissertation, Ch. Ismailova presents a total of 3 scientific publications in refereed and indexed journals in the world-renowned databases of scientific information (Web of Science and Scopus) - 2 articles in journals with Q3 and 1 article in a journal with Q4. These publications fulfil and exceed the Minimum National Requirements for the award of the educational and scientific degree Doctor, providing 42 points under Group G indicators, thereby surpassing the required minimum of 30 points, in accordance with the Regulations for the Implementation of the Act on the Development of the Academic Staff in the Republic of Bulgaria (last amended, State Gazette No. 16/10 February 2026), Professional Field 4.3 Biological Sciences.
In addition, the doctoral candidate has presented her research at two international scientific meetings and eleven national scientific conferences and has participated in three research projects directly related to the dissertation.

FINAL CONCLUSION

The dissertation submitted by **Chiydem Baarieva Ismailova** addresses a highly relevant and significant topic concerning an important human pathogen for which comprehensive epidemiological and genetic data in Bulgaria remain limited.
The research has been conducted using contemporary methodological approaches and demonstrates a high scientific quality. The dissertation is well organized, clearly written, and presented in precise scientific language.
Through this research, the doctoral candidate provides valuable new data on the sero-prevalence and molecular epidemiology of hepatitis E virus in Bulgaria, thereby making a meaningful contribution to the laboratory's long-standing research tradition in the field of hepatotropic viruses.

The dissertation fully complies with the mandatory requirements and scientometric criteria of the Act on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its Implementation (last amended, State Gazette No. 16/10 February 2026).
Based on the scientific merits of the dissertation, I give it a positive evaluation and strongly recommend that the esteemed Scientific Jury award Chiydem Baarieva Ismailova the educational and scientific degree of Doctor in the scientific specialty Virology, Professional Field 4.3 Biological Sciences, Field of Higher Education 4. Natural Sciences, Mathematics, and Informatics, professional field 4.3. Biological sciences; scientific specialty: Virology.

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/Prof. Neli Korsun, MD, DSc/