

To
The Chairperson of the Scientific Jury,
appointed by Order No. 182 of 30.06.2026 of
the Director of the National Center
of Infectious and Parasitic Diseases, Sofia.

With reference to your Protocol No. 1/07.07.2026, please find enclosed:

OPINION

for the conduct of a procedure for the public defense of a dissertation for the award of the educational and scientific degree "Doctor" in the Higher Education Field: 4. Natural Sciences, Mathematics and Informatics; Professional Field: 4.3. Biological Sciences; Scientific specialty: Virology, by Chiydem Baarieva Ismailova– biologist, Department of Virology, National Reference Laboratory "Hepatitis Viruses," National Center of Infectious and Parasitic Diseases.

Form of doctoral study: full-time doctoral candidate. Department of Virology, National Reference Laboratory "Hepatitis Viruses," National Center of Infectious and Parasitic Diseases.

Topic: "Studies on the Serological and Molecular-Genetic Characteristics of the Hepatitis E Virus"

Scientific supervisor: Assoc. Prof. Lubomira Nikolaeva-Glomb, MD, PhD

Scientific consultant: Chief Assistant Professor Elitsa Nikolaeva Golkocheva-Markova, MD, PhD

Opinion by Prof. Yordanka Dimitrova Stoilova, MD, PhD, external member

(ID card name: Milcheva)

Scientific specialty: Epidemiology

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The dissertation was prepared in the Department of Virology, NCIPD, Sofia.

General presentation of the procedure and of the doctoral candidate

The set of materials, in both hard copy and electronic form, complies with the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for the Implementation of the ADASRB, and the requirements of the Scientific Council of NCIPD, Sofia, and includes in full the documents

required for the defense, including a list of the activities carried out under the tasks of the doctoral candidate's individual study plan. The dissertation was discussed, the candidate was removed from the doctoral register, and the work was directed toward defense on 23.08.2023.

Presentation of the doctoral candidate, with emphasis on the biographical data relevant to the procedure

In 2019 she graduated from Sofia University "St. Kliment Ohridski," obtaining a Bachelor's degree in Molecular Biology, followed by a Master's degree in Microbiology and Microbiological Control. Since 2018 she has worked at NCIPD, Sofia, with core duties including: sample processing; work with a range of laboratory diagnostic methods; serological methods; the ELISA method; immunoblot assays; molecular methods – isolation of viral nucleic acids, polymerase chain reaction (PCR); and participation in scientific congresses.

Molecular biology of prokaryotic and eukaryotic microorganisms; antibiotics and antibiotic resistance; food microbiology; environmental microbiology.

The doctoral candidate's drive to train in a variety of directions, acquiring core theoretical knowledge and practical skills, is impressive and commendable:

- Participation in the 8th Meeting of the European Hepatitis B and C Network, organized by the European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden.
- Luxembourg Institute of Health (LIH) – sequencing and phylogenetic analysis.
- The National Center of Infectious and Parasitic Diseases (NCIPD) – laboratory diagnostics of viral infections; cultivation of viruses and rickettsiae under laboratory conditions; application of next-generation sequencing in clinical microbiology and the diagnosis of infectious diseases; general immunology and allergology.

Very good command of English and Spanish.

Relevance of the topic and appropriateness of the stated aims and tasks

The significance and relevance of the problem addressed in the scientific material stem from the study's focus on the theoretical and practical aspects of the diagnosis, epidemiology, clinical presentation, prevention, surveillance, and control of viral Hepatitis E. It should be noted that the infection remains insufficiently studied in Bulgaria, a fact underscored by its having been subject to mandatory reporting only since 2019, which makes it possible to establish a year-on-year increase in reported cases. Globally, the number of newly infected individuals is likewise rising every year, against a background of incompletely clarified features of the infection relating to regional differences in sources (HEV being the only hepatitis virus with an animal reservoir), transmission mechanisms, and the clinical course of acute and chronic HEV infection among vulnerable groups in endemic and non-endemic regions. It should be particularly noted that HEV is unique in that its different genotypes have distinct geographic distributions, sources of infection, and patient profiles.

In this respect, the present dissertation extends research on the theory and practice of viral Hepatitis E in Bulgaria through the study of seroprevalence in various populations and of the genetic characteristics of the virus. The results obtained enrich the problem from multiple angles, which represents a genuine challenge.

The aim and tasks of the dissertation by Chiydem Baarieva Ismailova are clearly formulated. Aim: to study the seroprevalence of hepatitis E and the molecular-genetic characteristics of HEV viral isolates for the period 2015–2023 in a heterogeneous population. The tasks, five in number, are correctly linked to the aim.

Command of the subject matter

Chiydem Baarieva Ismailova demonstrates thorough knowledge of HEV, covering its history, genetic diversity and evolution, morphology, physicochemical properties, viral genome, viral proteins and their functions, viral replication, genotypes, and subtypes. The doctoral candidate presents the most recent data summarizing the epidemiology (key structural indicators), pathogenesis, clinical manifestations of acute and chronic hepatitis E, diagnostics based on immunoenzymatic and molecular-biological methods, treatment, and non-specific and specific preventive measures. I may summarize that the doctoral candidate has produced a very thorough review of the scientific and practical status of the problem, fully consistent with the aim and the substance of the tasks set in the dissertation.

The list of 194 publications, of which 11 are in Cyrillic script and 138 in Latin script, forms the basis of the 21-page literature review. All are drawn upon in the presentation of the main sections of the work.

The dissertation comprises 116 pages, of which 8 are appendices. The work is illustrated with 30 figures and 24 tables.

Personal Research Contribution

Research methodology

The methodological approach allows the stated aim to be achieved and provides an objective answer to the tasks of the dissertation; it draws on a wide range of research methods. Over 17 pages, the doctoral candidate presents in detail the object and subject of observation, the units of observation (logical and technical), the observed variables, the stages of the study, and its administration.

The methods are precisely chosen, up to date, and informative, supported by a total of 13 tables and 2 figures.

1. Immunoenzymatic methods – enzyme-linked immunosorbent assay (ELISA) for the detection of specific antibodies, anti-HEV IgM/anti-HEV IgG. Anti-HEV IgM/IgG were determined using qualitative immunoenzymatic kits – anti-HEV IgM/anti-HEV IgG ELISA of the commercial brand Dia.Pro, Italy. Test principle: according to the manufacturer, the tests are characterized by 100% sensitivity and $\geq 95\%$ specificity.
2. Molecular-biological methods: extraction of viral RNA; real-time reverse-transcription polymerase chain reaction (real-time RT-PCR) for determination of viral load.
3. Reverse transcription and amplification of complementary DNA (cDNA) from a specific region of the HEV viral genome.
4. Nested PCR.
5. Detection of the amplified products.
6. Sequencing.

I hold in high regard the critical analysis presented as a method, identifying the main reasons for the spread of HEV. A total of 2,467 samples were processed (whole blood and serum from both patients and healthy individuals, drawn from various populations and healthcare facilities across the country), obtained at the National Reference Laboratory

“Hepatitis Viruses” of NCIPD. The processing and storage of the study materials were carried out with precision. The mandatory sample-processing procedures are described in detail, for both prospective and retrospective serum samples. The interpretation of the results obtained is consistent with the recommendations of the European Association for the Study of the Liver (EASL, 2018).

Statistical analysis of the results

The data were processed using Microsoft Office Excel 2010. A descriptive statistical analysis was performed to determine mean age, using descriptive statistics (25th and 75th percentiles). Frequency distributions (sex, seroprevalence) were calculated as percentages. Statistical significance (p-value) was determined using the online Epitools Epidemiological Calculators (Ausvet).

Epitools Epidemiological Calculators. Ausvet. Available at: <http://epitools.ausvet.com.au>

Characteristics and evaluation of the dissertation and its contributions

The results are described and analyzed in detail, in line with the tasks set. The research conducted demonstrates genuine scientific work, enabling Chiydem Baarieva Ismailova to formulate specific, detailed conclusions – eight in total – of significance for both theory and practice. The doctoral candidate concludes that Hepatitis E virus is an important etiological agent of acute hepatitis in Bulgaria – 31% overall, 36% in the prospective group and 17% in the retrospective group, based on the seroprevalence found among the 2,467 individuals studied. She emphasizes that, owing to the short-lived viremia, detection of HEV RNA in patient serum is possible only in the early stages of infection; a negative molecular result does not exclude the presence of acute infection. A high anti-HEV IgM value (serological index ≥ 10) indicates a possible presence of HEV RNA in the sample tested.

The findings reveal regional data – the genotype circulating in Bulgaria is HEV genotype 3, characteristic of Europe. The most common subtypes are 3e (48%), 3f (24%), and 3c (12%). Heterogeneity among the variants circulating in Bulgaria is noted.

Of practical value is the age structure established in the study, with those affected predominantly over 50 years of age. The results indicate that the male sex carries an increased risk of symptomatic HEV infection.

I accept the four contributions formulated by the doctoral candidate:

1. A correlation has been established between the serological index (anti-HEV IgM value) and the molecular marker (HEV RNA), providing a basis for establishing a diagnostic algorithm for confirming acute HEV infection.
2. Genetic heterogeneity has been established among the HEV isolates studied in Bulgaria, forming a basis for further phylogenetic study of the viral variants circulating in the country.
3. Unidentified viral variants have been identified, providing a basis for further genetic investigation.
4. This study also confirms that sex, age, occupation, and risk factors are relevant to the spread of HEV. Identifying risk groups is of key importance for improving prevention and health surveillance in the country.

I agree with the doctoral candidate's statement that the results and contributions are original and not drawn from studies or publications in which she did not participate, and that the information contained in the documents, references, and publications required for the procedure is accurate and corresponds to objective fact.

The results obtained, together with the conclusions and contributions made, provide a basis for the development and refinement of future national standards for good medical practice and for the surveillance and control of HEV infection.

Publication record

Chiydem Baarieva Ismailova has submitted a list of a total of 5 scientific papers related to the dissertation, totaling 42 points, which exceeds the required 30 points under indicator group G of the Minimum National Requirements set out in the Regulations for the Implementation of the ADASRB – Section II, Conditions and Procedure for Acquiring the Educational and Scientific Degree “Doctor” (last amended, State Gazette No. 16 of 10.02.2026; Field 4. Natural Sciences, Mathematics and Informatics; Professional Field 4.3. Biological Sciences). The candidate’s points are based on 3 scientific publications indexed in Web of Science and Scopus, of which 2 are in Q3 journals and 1 in a Q4 journal; she is first author on 2 articles and second author on 1. She is first author in 8 of her 12 participations in scientific forums, three of which were abroad.

The doctoral candidate’s participation is active and of considerable scientific value in the areas of genotyping and diagnosis of hepatitis E in swine and food products, within 3 scientific projects related to the dissertation.

The autoreferat (dissertation abstract), comprising 68 pages (including 17 figures and 20 tables), is structured in accordance with the requirements, with content and quality that correctly and logically reflect the results achieved in relation to the aim and tasks of the work and its principal conclusions.

CONCLUSION

The dissertation contains scientific, applied-scientific, and applied results that constitute an original contribution to science and meet all the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for the Implementation of the ADASRB, and the corresponding Regulations of NCIPD, Sofia.

The dissertation shows that the doctoral candidate Chiydem Baarieva Ismailova possesses thorough theoretical knowledge and professional skills in the scientific specialty “Virology” and demonstrates the qualities required to conduct independent scientific research. The work is topical and carries substantial applied diagnostic, clinical, and epidemiological value for everyday medical practice. In view of the foregoing, I confidently give my positive assessment of the research, dissertation, autoreferat, results, and contributions reviewed above, and I propose to the esteemed Scientific Jury that the educational and scientific degree “Doctor” be awarded in the Higher Education Field 4. Natural Sciences, Mathematics and Informatics; Professional Field 4.3. Biological Sciences; scientific specialty Virology.

03.08.2026

Prof. Yordanka Stoilova, MD, PhD

