

OPINION

**by Assoc. Prof. Dr Rainichka Petrova Mihaylova-Garnizova,
Head of the Department of Infectious Diseases – Military Medical Academy,
Sofia**

on a doctoral thesis for the award of the academic and scientific degree of **‘Doctor’**
professional field 7.1. Medicine
Doctoral programme: Virology

Author: Chidem Baarieva Ismailova

Type of doctoral programme: full-time

**National Centre for Infectious and Parasitic Diseases – Department of
Virology**

Topic: Studies on the serological and molecular genetic characteristics of the
hepatitis E virus

Supervisor: Assoc. Prof. Dr Lyubomira Nikolaeva-Glomb, MD

Scientific consultant: Senior Assistant Professor Elitsa Nikolaeva Golkochova-
Markova, MD

1. General overview of the procedure and the PhD candidate

**1.1 The set of materials submitted on electronic media complies with the
Procedure for the Award of the Academic Degree of ‘Doctor’; documents:**

- **A doctoral thesis comprising 116 pages, of which 89 are main text**
- **An abstract of 67 pages**
- **A CV in European format with an appendix entitled ‘Research Activity up to 2025’**
- **A copy of the bachelor’s and master’s degree certificates**
- **Copies of publications related to the topic of the doctoral thesis**

ECDC), Stockholm, Sweden. Training in sequencing and phylogenetic analysis in 2023 at the Luxembourg Institute of Health. Application of next-generation sequencing in clinical microbiology and the diagnosis of infectious diseases, 2021, ESCMID

Further training at the National Centre for Infectious and Parasitic Diseases (NCIPD): Laboratory diagnosis of viral infections; Cultivation of viruses and rickettsiae in laboratory conditions; and General immunology and allergology.

Additional qualifications: proficiency in English (C1) and Spanish (B2).

2. Relevance of the topic

The topic of the thesis is of current relevance. It stems from the experience gained in clinical practice over the last 40 years relating to hepatitis E virus infection. The topic is significant because it is frequently a missed diagnosis in clinical practice, as this infection is not usually considered. In many cases, it is diagnosed in patients with cancer or those who are immunocompromised during a more extensive diagnostic investigation.

3. Characterisation and evaluation of the thesis and its contributions

The thesis comprises 116 pages and contains 24 tables and 30 figures. The bibliography includes 301 references, of which 14 involve Bulgarian research teams. 274 (91 per cent) of the references are from the last 10 years, and 255 (84.7 per cent) from the last 5 years.

It is structured in accordance with the accepted requirements and the Regulations of MU and contains: an introduction, a literature review, aim and objectives, materials and methods, results, discussion, conclusions, contributions, publications and a bibliography. The author has noted the contributions and publications related to the thesis

The literature review (29 pages) is based on a bibliographic survey of 195 titles. It provides a general overview of (1) hepatitis E viral infection, (2) the aetiology and structure of the virus, (3) pathogenesis, (4) diagnosis and (5) treatment.

The review is informative and demonstrates the doctoral candidate's excellent knowledge of the current state of the field. The critical analysis of the review outlines the problems and justifies the objectives of the thesis.

The aim and objectives are precisely formulated. The aim corresponds to the topic. The objectives specify the directions of the study.

Materials and methods.

A total of 2,467 samples (whole blood and serum from patients and healthy individuals from various populations and different healthcare facilities across the country) were processed at the National Reference Laboratory for 'Hepatitis Viruses' at the National Centre for Infectious and Parasitic Diseases. Of these, 1,823 were prospective serum samples from hospitalised patients with various diagnoses and 644 were retrospective serum samples from a heterogeneous population, collected between 2015 and 2023.

A wide range of methods were used: Immunoenzymatic methods, molecular biological methods (extraction of viral RNA, real-time RT-PCR, reverse transcription and amplification of cDNA from a specific region of the HEV viral genome, nested PCR, detection of amplified products), sequencing, as well as statistical methods for analysing the results obtained.

The use of various standardised statistical methods is noteworthy

Results and Discussion

The results are clearly presented, organised in tables and, last but not least, a well-formulated cladistic analysis of the obtained HEV sequences. The conclusions are entirely derived from the results obtained. They are comprehensively formulated, and the hepatitis E virus is identified as an important aetiological agent of acute hepatitis in Bulgaria, with seroprevalence among the 2,467 individuals studied at 31 per cent, with 36 per cent in the prospective group and 17 per cent in the retrospective group. It is confirmed that genotype 3 of HEV, characteristic of Europe (), is circulating in the Republic of Bulgaria, with the most common subtypes being 3e (48%), 3f (24%) and 3c (12%).

I agree with the contributions outlined by the PhD student. Of particular significance to me is the established correlation between the serological index (anti-HEV IgM level) and the molecular marker (HEV RNA), which forms the basis for establishing a diagnostic algorithm to detect acute HEV infection, confirming the genetic heterogeneity of the HEV isolates studied, whilst the unidentified variants of the virus, which will be investigated in the future.

The thesis makes undoubted contributions and is of significant importance to science and practice.

Assessment of the doctoral candidate's publications and personal contribution

The thesis is the doctoral candidate's own work, as is the formulation of the conclusions and contributions.

The research projects related to the thesis are:

1. 'Studies on genotypic diversity and the role of the polyproline region in the in vitro adaptation of the hepatitis E virus', contract No. KP-06-N33/2 of the National Science Fund
2. 'Improving national diagnostic capabilities for the detection of the hepatitis E virus in pigs and pig-derived products' (BUL5017), funded by the International Atomic Energy Agency (IAEA)
3. Project No. BG05M2OP001-1.002-0001, funded by the Operational Programme 'Science and Education for Smart Growth', co-financed by the European Union through the European Regional Development Fund

The abstract has been prepared in accordance with the requirements and reflects the main results achieved in the thesis

Critical comments and recommendations

I recommend increasing publication output, including in international journals and those with an Impact Factor.

Conclusion

The thesis contains scientific-applied and applied results which represent an original contribution to science and meet all the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria (LDASRB), The materials presented and the results of the thesis fully comply with the specific requirements set out in the Regulations of the National Centre for Infectious and Parasitic Diseases (NCIPD) for the implementation of the ZRASRB.

The doctoral thesis demonstrates that the doctoral candidate, Chidem Baarieva Ismailova, possesses in-depth theoretical knowledge and practical skills in virology,

whilst demonstrating the qualities and abilities required to conduct independent scientific research.

In view of the above, I am confident in giving my positive assessment of the research presented in the doctoral thesis described above, abstract, results achieved and contributions, and I propose that the distinguished academic jury award the academic and scientific degree of 'Doctor' to Chidem Baarieva Ismailova in the doctoral programme in virology

12 August 2026

Opinion prepared by:

Assoc. Prof. Dr Rainichka Petrova Mihaylova-Garnizova