To
The Chair of the Scientific Jury
Assoc. Prof. Ivan Baltadzhiev, MD, PhD
Appointed by Order No. R-5191/17.09.2026
of the Rector of Medical University - Plovdiv

ACADEMIC OPINION

By Prof. Ani Kevork Kevorkyan, MD, PhD
Head of the Department of Epidemiology and Disaster Medicine
Faculty of Public Health, Medical University – Plovdiv

concerning

a procedure for the defense of a dissertation for the award of the educational and scientific degree "Doctor" in higher education area 7. Healthcare and Sports, professional field 7.1. Medicine, doctoral programme "Infectious Diseases", at the Department of Infectious Diseases, Parasitology and Tropical Medicine, Medical University - Plovdiv.

Author of the dissertation: Dr. Stanislav Vasilev Kotsev
Topic: "Prevalence and Clinical Characteristics of Hepatitis E Virus Infection among Individuals from Risk Groups during the Period 2018–2022"
Scientific supervisors: Prof. Oliana Boykinova, MD, PhD, and Prof. Nikolay Vatev, MD, PhD

1. Description of the submitted documents and materials: The documents and materials submitted for the procedure for awarding the educational and scientific degree "Doctor" meet the requirements of the Regulations for the Implementation of the Act on the Development of the Academic Staff in the Republic of Bulgaria and the corresponding Regulations of Medical University – Plovdiv, providing the supporting documentation necessary for conducting the procedure. I have no joint publications or research projects with the doctoral candidate.

2. Presentation of the doctoral candidate (professional and academic development):
Dr. Stanislav Vasilev Kotsev was born in 1992. He graduated from Bertolt Brecht Foreign Language High School in Pazardzhik and Sofia University "St. Kliment Ohridski", where he obtained his Master's degree in Medicine in 2017. Since graduation, he has worked, and continues to work, in the Infectious Diseases Department of Pazardzhik General Hospital. From October 2025 to February 2026, he was a part-time teaching assistant at the Department of Zoology, Faculty of Biology, Paisii Hilendarski University of Plovdiv, teaching "Vectors in Vector-Borne Infections". He obtained his specialty qualification in Infectious Diseases in 2022.
He was enrolled as an independent-study doctoral candidate in the "Infectious Diseases" doctoral programme at the Department of Infectious Diseases, Parasitology and Tropical Medicine, Medical University – Plovdiv (Order No. R-643/22.02.2024), with the dissertation topic "Prevalence and Clinical Characteristics of Hepatitis E Virus Infection among Individuals from Risk Groups during the

Period 2018–2022", and subsequently completed his doctoral training with the right to defend (Order No. R-3378/05.06.2026).

The doctoral candidate is proficient in English (C1 level), has a working knowledge of German, and possesses very good computer skills, which are prerequisites for a successful scientific career. Dr. Kotsev makes continuous efforts to improve his professional qualifications. Between 2021 and 2026, he attended seven courses, four of them abroad: Medical Experts Forum (Vienna, Austria, 2022), BREATHE 2023 International Summit (Windsor, United Kingdom), BREATHE 2024 International Summit (Madrid, Spain), and the Open Medical Institute Weill Cornell Seminar in Infectious Diseases (Salzburg, Austria, 2026).

His extensive project activity is noteworthy, covering important topics in infectious disease pathology. Dr. Kotsev has participated, or is currently participating, in two national and three international projects, despite not being employed by a research institution or university: "Immunological Memory in SARS-CoV-2/COVID-19: Mechanisms, Duration and Cross-Reactivity"; "Monkeypox in Bulgaria – Clinical Course and Route of Transmission"; "Analyzing C. difficile Infections"; "Hospital Acquired Blood Stream Infections"; and "Vector-Borne and Zoonotic Infections Survey".

He belongs to seven scientific organizations: the Bulgarian Society of Infectious Diseases; the Bulgarian Association of Microbiologists; the Southern Bulgarian Society of Infectious Diseases, Epidemiology and Parasitology (member of the Management Board); the Bulgarian Association of Preventive Medicine; Infectious Diseases – International Research Initiative (IDI-IRI); the European Society of Clinical Microbiology and Infectious Diseases (ESCMID); and the Balkan Association for Vector-Borne Diseases. He is also a member of the professional organization, the Bulgarian Medical Association (BMA).

During the COVID-19 pandemic, Dr. Kotsev received regional and national recognition for his professionalism and clinical skills, receiving the BMA award "You Are Our Future".

3. Relevance of the topic: Viral hepatitis E is a disease that continues to attract the interest of researchers worldwide more than 40 years after its causative agent was discovered and isolated. It is rightly described as an infection with "two faces", differing primarily in their epidemiological and, to some extent, clinical characteristics, associated with the predominant genotypes of hepatitis E virus (HEV).

According to the World Health Organization (WHO), 20 million people become newly infected each year, of whom only 3.3 million (16.5%) develop clinically apparent disease. Data compiled by the European Centre for Disease Prevention and Control (ECDC) in recent years, beginning in 2011, indicate a substantial increase in new cases, most of them autochthonous. Certain parts of southern France, Poland and Denmark show a very high prevalence of anti-HEV IgG in the general population, encouraging the characterization of regional disease profiles. Research continues into the reservoirs and sources of infection, groups at risk of infection or severe disease, clinical characteristics and extrahepatic manifestations, the risk of chronic infection in immunocompromised individuals, the safety of donated blood and the need for its screening, among other issues.

Hepatitis E is not a new infection in Bulgaria. The first cases described and the first publications in this country date back to 1995, the year in which HEV genotype 3 (characteristic of pigs) was isolated. Their number has increased over time owing to growing interest in the disease and the availability of diagnostic tests. In an effort to characterize hepatitis E, individual aspects of the epidemiology and clinical course of HEV infection have been studied in several regions, primarily Plovdiv and Pazardzhik. In 2019, Dr. M. Pishmisheva presented the first comprehensive study of acute HEV infection in her dissertation, "Clinical and Epidemiological Aspects of Acute Hepatitis E Virus Infection in Pazardzhik Region".

4. Knowledge of the problem: The dissertation submitted to me for review, prepared by Dr. Stanislav Kotsev, represents a logical continuation and further development of hepatitis E research. Its

focus is on topics that have received little or no attention, such as the prevalence and clinical characteristics of infection among at-risk groups, including patients with underlying liver disease, immunosuppressed patients, patients receiving chronic hemodialysis, people who abuse alcohol, and blood donors.

All this makes the study, supported by a wide range of modern laboratory methods, important and timely. It not only attempts to answer some disputed questions but also has practical value, as it is directly related to defining the regional characteristics of HEV infection in Pazardzhik Region. It may also serve as a model for conducting in-depth clinical and epidemiological studies in other regions of the country.

My overall impression is that the doctoral candidate has an in-depth understanding of the problem. He discusses and analyzes its various aspects equally well in both the literature review and his original studies. This is also facilitated by his daily work as a physician in the Infectious Diseases Department, direct contact with patients, the opportunity to obtain detailed epidemiological histories, and the ability to follow patients over time.

5. Characteristics and evaluation of the dissertation/dissertation abstract, conclusions and contributions: In its present form, the dissertation is a completed work comprising 138 standard pages, illustrated with 39 figures and 28 tables. It follows the generally accepted structure: introduction (1 page), literature review (28 pages), aim and objectives (1 page), materials and methods (6 pages), results (38 pages), discussion (12 pages), algorithm for testing donated blood (1 page), conclusions (1 page), contributions (2 pages), recommendations (1 page), and appendices (16 pages). The bibliography contains 403 sources, 37 in Bulgarian and the remainder in English. Bulgarian research teams that have contributed to the study of HEV infection in Bulgaria are duly acknowledged. The literature review is precisely focused on the dissertation topic and is well structured. The information is presented in depth, specialist terminology is used appropriately, and the cited works are directly relevant to the subject. The review follows a logical sequence, beginning with the first detailed description in Kashmir, India, and the features of this previously "unknown" disease—including its route of transmission, the age of those affected and prognosis in pregnant women—and proceeding to the identification of its etiology. Of interest is the information that the four main HEV genotypes infecting humans developed over a period of 500 years and that, as living conditions improved in Europe, HEV 1 and 2 "shifted" toward the Middle East and Africa (developing countries), while HEV 3 and 4 continued to circulate in developed countries. This changes our understanding of the supposedly exotic origin of HEV and its introduction into Europe from Asia. The etiology is described in detail, providing additional in-depth information on the genera within the family Hepeviridae and their animal reservoirs, as well as the possible role of quasi-enveloped virions in extrahepatic manifestations of HEV infection. A substantial part of the review addresses established and uncertain mechanisms of transmission, the risks of infection and severe disease in particular population groups, and the characteristics of the epidemic process in areas with different levels of endemicity. Sufficient attention is also devoted to pathogenesis, pathology, clinical manifestations, complications, extrahepatic manifestations, diagnosis, treatment, and control and prevention measures. I consider that the literature review, as presented, has considerable educational and practical value. Its analysis also defines the scope of the original studies in the dissertation.

The aim is clearly and specifically formulated: to investigate the prevalence and clinical characteristics of hepatitis E virus infection among individuals from risk groups (those with underlying liver disease, immunocompromised individuals, and alcohol users) and individuals without risk factors (members of the general population and blood donors) during 2018–2022. Five main objectives were established to achieve this aim. Their formulation follows logically from the literature review: to investigate the prevalence of HEV infection among at-risk groups in Pazardzhik Region; to investigate its prevalence among individuals without risk factors—the general population and blood donors; to investigate the

clinical characteristics of HEV infection among at-risk groups; to compare the prevalence and clinical course of HEV infection in individuals with and without associated risk factors; and to propose an algorithm for HEV testing of donated blood and recommendations for testing individuals from risk groups.

The "Materials and Methods" section is detailed and well structured, accurately reporting the number of participants examined for each objective, divided into two groups (individuals with risk factors and individuals without risk factors), as well as the clinical specimens tested. A variety of methods were applied: epidemiological investigation, clinical methods, questionnaire surveys, clinical laboratory methods, diagnostic imaging, serological methods, and molecular biological methods. The statistical methods ensure a high degree of reliability of the research and, accordingly, of the conclusions drawn.

In the original research section, "Results" and "Discussion" are presented separately and together comprise 51 pages. The study covers five calendar years (2018–2022) and includes 616 adults from Pazardzhik Region, divided into two main groups:

1. Individuals with risk factors for HEV infection and severe disease – 371, distributed into the following subgroups: underlying liver disease – 98; immunosuppression – 78; end-stage renal failure receiving chronic hemodialysis – 102; more than one risk factor (underlying liver disease and immunosuppression) – 73; and alcohol abuse – 20.
2. Individuals without risk factors – 245, including 151 members of the general population without associated risk factors and 94 blood donors at the Department of Transfusion Hematology of Pazardzhik General Hospital in 2021.

Some of the most important findings from the original studies are the high prevalence of serological markers of hepatitis E among both at-risk groups and the general population and blood donors. Such markers were found in 34% of all those examined (245/616), predominantly older (>55 years) men of Bulgarian origin. Individuals from risk groups were more frequently affected, especially those with underlying liver disease. These characteristics were also confirmed in patients with clinically apparent hepatitis E. Immunosuppression was associated with a longer preicteric period and anicteric hepatitis. The leading risk factor for clinically apparent icteric hepatitis E was underlying liver disease. Liver cirrhosis, regardless of etiology, was associated with severe illness and complications, the development of acute-on-chronic liver failure, and death. The case fatality rate reached 6.8%, with deaths occurring during acute or acute-on-chronic liver failure. HEV infection in risk groups requires targeted testing, careful interpretation of diagnostic results, and heightened clinical vigilance, especially when diagnosing extrahepatic manifestations.

Five principal conclusions were formulated, accurately summarizing the results of the studies conducted for the dissertation. I agree with the doctoral candidate's self-assessment of the work's contributions (three contributions original to Bulgaria, two confirmatory contributions, and three contributions of scientific and practical significance). I shall focus only on the original contributions:

1. For the first time in Pazardzhik Region, the prevalence of hepatitis E virus infection has been investigated in groups at risk of infection and severe disease (underlying liver disease, immunosuppression, alcohol abuse, and chronic renal failure requiring chronic hemodialysis).
2. For the first time in Bulgaria, the clinical features of hepatitis E virus infection among individuals from risk groups have been investigated in detail, including clinical manifestations, laboratory abnormalities, extrahepatic manifestations, clinical course, complications, and outcomes.
3. For the first time in Bulgaria, the prevalence of hepatitis E virus infection among blood donors from Pazardzhik Region has been investigated, and results of molecular biological testing of donated blood for HEV RNA have been presented, together with a proposed testing algorithm.

The dissertation abstract is 60 pages long, is very well presented, and is sufficiently informative, reflecting the content, main findings and contributions of the dissertation.

6. Evaluation of publications and participation in scientific conferences/forums on the doctoral topic: The doctoral candidate has provided supporting evidence of four full-text publications on the dissertation topic, including one in a scientific journal indexed in Scopus, in quartile Q4: *"PROBLEMS of Infectious and Parasitic Diseases*" (2024). The remaining articles were published in the *"International Journal of Current Microbiology and Applied Sciences (IJCMAS)"* (2020), *"Medinfo"* (2023), and *"Preventive Medicine"* (2022). Dr. Kotsev is first author of two articles. Some of the dissertation findings have been presented and discussed before a broad range of specialists working in infectious diseases (microbiologists, infectious disease specialists, virologists, immunologists and epidemiologists), pediatricians, and others. This is evidenced by participation in a total of four national scientific forums. I consider that the submitted publications and conference participation accurately reflect the doctoral candidate's achievements and help disseminate them within the scientific community.

7. Recommendations: The doctoral candidate has made additions to the final version of the dissertation in accordance with the recommendations made during the internal defense.

Conclusion: Dr. Stanislav Kotsev is a young and promising infectious disease physician and researcher. He possesses in-depth theoretical knowledge and professional skills in the scientific specialty "Infectious Diseases" and demonstrates the qualities and abilities required to conduct independent scientific research. This is evidenced by the well-structured, carefully described and analyzed original studies in the dissertation, as well as by his accumulated clinical experience. The dissertation contains scientific-theoretical and scientific-applied results representing original contributions in Bulgaria and meets all the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria and the specific requirements adopted in the regulations of Medical University – Plovdiv.

On the basis of the foregoing, I give a positive assessment of the research presented in the dissertation, dissertation abstract and publications under review, and I strongly support the award of the educational and scientific degree "Doctor" in the doctoral programme "Infectious Diseases" to Dr. Stanislav Vasilev Kotsev (physician in the Infectious Diseases Department of Pazardzhik General Hospital).

Заличено на основание
чл.5 §1, б."в" Регламент (ЕС)2016/679

Opinion prepared by:

Prof. Ani Kevorkyan, MD, PhD

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