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REVIEW

by Assoc. Prof. Ivan Georgiev Baltadzhiev, MD, PhD

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Chairperson of the Scientific Jury, appointed by Order No. R-5191/17 September 2026 of the Medical University of Plovdiv, and acting on the basis of Minutes No. 1 of the first meeting of the Scientific Jury, held on 18 September 2026.

Regarding: the dissertation of Dr. Stanislav Vasilev Kotsev entitled "Prevalence and Clinical Characteristics of Hepatitis E Virus Infection among Individuals from Risk Groups during the Period 2018–2022", submitted for the educational and scientific degree of Doctor (PhD) in the doctoral programme Infectious Diseases, professional field 7.1 Medicine.

Scientific supervisors: Professor Oliana Boykinova, MD, PhD, and Professor Nikolay Vatev, MD, PhD.

Brief biography of the doctoral candidate

Dr. Stanislav Vasilev Kotsev graduated in medicine in 2017 from the Faculty of Medicine of Sofia University "St. Kliment Ohridski". His professional career has been closely associated with infectious diseases and clinical practice at Pazardzhik Multiprofile Hospital for Active Treatment AD. Between April 2018 and May 2022, he worked as a resident in infectious diseases, obtaining his specialist qualification at Medical University – Plovdiv in May 2022. Since June 2022, he has worked as a specialist in infectious diseases in the Infectious Diseases Department of Pazardzhik Multiprofile Hospital for Active Treatment AD.

His research interests focus on infectious diseases, viral hepatitis, epidemiology and the diagnosis of infections. He actively participates in national and international scientific forums and is a member of professional organisations, including the Bulgarian Society of Infectious Diseases and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). His submitted curriculum vitae also mentions participation in a research project on immunological memory in SARS-CoV-2/COVID-19 during 2020–2023.

Between October 2025 and February 2026, he worked as a part-time teaching assistant at the Faculty of Biology of Paisii Hilendarski University of Plovdiv, teaching "Vectors in Vector-Borne Infections". His professional and scientific activities are well documented through publications, conference participation and specialised training. In 2020, he received the "You Are Our Future" award from the Bulgarian Medical Association at both national and regional levels. These facts demonstrate consistent professional development in infectious diseases and confirm that his clinical interests fully correspond to the dissertation topic.

1. General characteristics and relevance

Viral hepatitis E occupies a distinctive position among infectious diseases affecting the liver. This reflects its multiple routes of transmission, diverse clinical spectrum and the considerable influence of the patient's general condition on prognosis. Studies of specific patient groups are therefore particularly important: they supplement general epidemiological evidence and help clinicians recognise the infection more readily in everyday practice.

In Europe, hepatitis E is no longer regarded solely as a disease associated with travel to endemic areas, but also as a zoonotic infection. Consequently, clinicians must remain vigilant even in patients with no history of foreign travel. The dissertation examines this issue in a regional context and links epidemiological observations to specific population categories.

The topic is well justified, particularly given the importance of differential diagnosis in acute hepatitis of unknown aetiology. By addressing serological markers, molecular biological methods and clinical manifestations together, the dissertation provides a comprehensive account of the infection.

The practical significance of the study is especially evident for patients with chronic liver disease, individuals undergoing haemodialysis and immunosuppressed patients. These groups differ in comorbidities, routes of infection and immune responses, making the comparative approach particularly valuable.

In summary, hepatitis E is characterised by a wide range of epidemiological mechanisms and clinical manifestations. In Europe, autochthonous cases and zoonotic transmission are critically important, while certain patient groups face a disproportionately high risk of severe disease. The choice of topic is scientifically well founded because it combines seroepidemiological and clinical analyses of considerable relevance to infectious disease practice.

Both serological and molecular biological methods are used to diagnose HEV infection. Combining anti-HEV IgM, anti-HEV IgG and HEV RNA provides a more complete picture of infection and supports more accurate clinical interpretation of findings.

The dissertation is particularly useful to physicians treating patients with chronic liver disease, patients on haemodialysis and individuals with immune disorders. In these groups, both the likelihood of infection and disease severity may differ from those in healthier individuals. It should be noted that the study's contribution relates to the selected sample and should not automatically be considered representative of the country as a whole.

The dissertation was prepared at the Department of Infectious Diseases, Parasitology and Tropical Medicine of Medical University – Plovdiv under the supervision of Professor Oliana Boykinova and Professor Nikolay Vatev. It comprises 138 pages, including 39 figures, 28 tables, 31 appendices and an extensive bibliography of 403 references.

The topic is highly relevant in view of the role of hepatitis E in acute liver injury, chronic diseases, immunodeficiency and transfusion medicine.

2. Aim, objectives and organisation of the study

The study combines two important perspectives: assessment of markers indicating exposure to the virus and description of its clinical manifestations. Epidemiological findings thus acquire direct practical relevance rather than remaining merely descriptive statistics.

Because the study population includes individuals with diverse risk factors, such as liver disease, immunosuppression, haemodialysis and alcohol misuse, it encompasses a broad clinical profile. Comparison with individuals without these risk factors provides a sound basis for analysing serological markers.

The use of ELISA to detect anti-HEV IgM and IgG is appropriate for evaluating the immune response, while the addition of RT-PCR for HEV RNA extends diagnostic capabilities by directly detecting viral genetic material.

The study covers 2018–2022, providing substantial data collected over several years. The combination of clinical, laboratory and epidemiological information allows the issue to be examined both from the perspective of infectious disease and that of associated comorbidities.

The principal aim is to investigate the prevalence and clinical characteristics of HEV infection in risk and non-risk groups during the specified period. Five objectives are defined, ranging from seroepidemiology to a proposal for a new blood donation algorithm.

The study included 616 adults: 371 with risk factors (including liver disease, immunosuppression, haemodialysis or combinations of risks) and 245 without such factors (outpatient clinic attendees and blood donors). This composition permits informative comparisons, although the participant selection process requires careful consideration.

The methodology encompasses epidemiological, clinical and molecular biological approaches. ELISA assays were used for antibody detection, and RT-PCR was used to identify viral RNA in selected cases. Statistical analysis included parametric and non-parametric tests and regression analyses. A major strength is the close integration of clinical data, laboratory findings and risk-factor assessment.

3. Assessment of the results and their scientific interpretation

The results across the different groups clearly demonstrate the diversity of exposure to HEV. Seropositivity indicates an immunological trace of infection, whereas the presence of viral RNA represents a different diagnostic dimension. Considering these markers together is highly useful in clinical practice.

The dissertation has a strong practical orientation because the same virus may manifest very differently in a healthy person and in a patient with pre-existing liver injury. Information about comorbidities is therefore central to this work.

Particular attention is devoted to immunosuppressed patients, adding substantial value to the study. In this group, antibody dynamics and the risk of viral persistence are critical clinical issues.

The inclusion of haemodialysis patients broadens the scope to a group with frequent healthcare contacts and specific needs. The regional findings provide a valuable addition to knowledge about HEV in this setting.

The inclusion of blood donors also extends the research into transfusion medicine. These observations contribute to the broader scientific discussion of HEV epidemiology and ways of limiting transfusion-transmitted infection.

According to the author, the highest reported proportion of anti-HEV IgG was 29.57% of those examined, with higher seropositivity in certain risk groups. The conclusions state that specific anti-HEV antibodies were detected in 40% of blood donors, but HEV RNA was not detected among these seropositive donors. These findings merit attention but should not be interpreted as a definitive estimate of the frequency of active viraemia or the national risk associated with transfusion.

Clinically, the study emphasises more severe disease in patients with concomitant liver disorders, particularly cirrhosis, and diagnostic challenges in immunocompromised patients. It also considers extrahepatic manifestations, which are highly important in differential diagnosis. Interpretation should distinguish associations from proven causal relationships and account for the effects of comorbidities.

4. Scientific and applied scientific contributions

The dissertation's principal contribution lies in the collection and systematic presentation of original data on the prevalence of HEV markers in specific clinical groups. The data come from a regional Bulgarian population and allow comparison with findings from other countries and populations.

Another important strength is the linkage between seroepidemiological findings and the characteristics of the clinical course. This makes the work useful not merely as a statistical account but also as valuable material for clinical diagnostic reasoning.

The discussion of HEV in chronic liver disease and immunosuppression draws attention to patients for whom timely recognition of infection is critically important. This is directly relevant to the specialty of infectious diseases.

Data on the various risk groups and on individuals without such risks also have educational value and can be used in teaching medical students, residents and physicians in infectious and internal medicine.

The scientific value of the dissertation is evident in its systematic presentation of HEV seroepidemiological data across different groups and their comparison at regional level.

From an applied scientific perspective, the contribution lies in enriching the diagnostic approach to hepatitis E in acute liver injury and in patients with increased clinical vulnerability.

The principal contributions include comparative investigation of different groups, clinical characterisation of HEV infection in the presence of comorbidities and discussion of its implications for transfusion safety.

The dissertation's practical value lies in directing clinical attention towards HEV in acute liver injury, including in patients with chronic liver conditions or immune disorders.

The scientific publications presented reflect the main lines of investigation and demonstrate the doctoral candidate's sustained work on HEV infection.

5. Publications, dissertation abstract and academic integrity

The publication record demonstrates an effort to disseminate the findings beyond the dissertation itself. The relationship between the study and the scientific materials produced is an important aspect of its academic evaluation.

The dissertation abstract systematically presents the main elements of the work, enabling readers to identify the research aim, methods, principal results and contributions.

Taken together, the dissertation, abstract and publications demonstrate consistent development of the topic and dissemination of findings in different academic formats.

The submitted documentation includes publications on the topic, including an article on HEV among haemodialysis patients in the Pazardzhik region (2020), a publication on prevalence among individuals who misuse alcohol (2022), and an article on extrahepatic manifestations (2024). These demonstrate active involvement in scientific publishing. A final assessment requires comparison of the complete publication list, authorship, indexing and the requirements of Medical University – Plovdiv.

Detailed assessment of the seroepidemiological results

Table 3 presents the distribution of seropositive individuals by sex and ethnicity. Among the 616 participants, 210 had specific anti-HEV antibodies, representing 34% of the sample. This proportion provides an important starting point for discussing the prevalence of HEV markers in the Pazardzhik region during the study period.

By sex, 145 of 379 men (38%) and 65 of 237 women (27%) were seropositive; the table reports a statistically significant difference ($p = 0.006$). This is an interesting demographic finding that enriches the description of the regional sample. The mean age was 55.57 ± 15.3 years among seropositive individuals and 53.76 ± 16.7 years among seronegative individuals, with no statistically significant difference.

Dividing the population according to the presence of risk factors allows a clearer comparison of serological results. Among 371 participants with risk factors, 129 (35%) were seropositive, compared with 81 of 245 (33%) without risk factors. The comparison in Table 14 gives $\chi^2(1) = 0.19$ and $p = 0.661$. Scientifically, this indicates that examining individual risk categories is more informative than simply dividing participants into two broad groups.

The epidemiological interpretation of these findings has important practical implications: HEV markers are also found in people outside the standard clinical risk groups. The dissertation thus demonstrates the significance of the infection beyond a specific hospital population and supports a broader public health perspective.

Comparative characteristics of the risk groups

The dissertation distinguishes groups with concomitant liver diseases, immunosuppressed patients, individuals receiving chronic haemodialysis, patients with more than one risk factor and individuals who misuse alcohol. The study population therefore encompasses different pathophysiological predispositions to infection and severe disease. This classification facilitates understanding of the specific characteristics of HEV infection in practice.

The results of the binary logistic regression analysis in Table 16 are particularly informative. Compared with the group without risk factors, the category of concomitant liver disease has an odds ratio (OR) of 2.11 (95% confidence interval 1.31–3.40; $p = 0.002$). This quantitatively demonstrates the association between this category and anti-HEV positivity in the study sample.

For individuals with chronic kidney disease receiving haemodialysis, the regression model reports OR = 0.32 (95% confidence interval 0.17–0.60; $p < 0.001$). For immunosuppression, OR = 1.07 (95% CI 0.63–1.83; $p = 0.8$), and for combined liver disease and immunosuppression, OR = 1.26 (95% CI 0.73–2.17; $p = 0.403$). Presenting these estimates alongside confidence intervals provides a sound basis for scientific interpretation.

The regression model is reported as $\chi^2(5) = 36.58$, $p < 0.001$, and Nagelkerke $R^2 = 0.08$, with $n = 616$. These parameters allow the reader to assess the main characteristics of the analytical approach. The dissertation therefore goes beyond describing percentages to provide quantitative estimates of associations between different categories and laboratory markers.

Assessment of the diagnostic and clinical components

One of the major strengths of the dissertation is its combination of serological and molecular biological testing. Anti-HEV IgG indicates previous immunological exposure, anti-HEV IgM helps identify recent infection, and HEV RNA detection directly demonstrates the presence of viral genetic material. These complementary diagnostic levels enrich the analysis and connect laboratory findings with the actual clinical picture.

Investigating HEV in patients with chronic liver disease is especially important because acute infection may be superimposed on pre-existing liver damage. The clinical section examines disease characteristics in patients with cirrhosis and other concomitant conditions. The dissertation thus highlights cases in which establishing the aetiological diagnosis is crucial to the patient's overall condition.

Immunosuppressed patients form a distinct group in whom the immune response may affect interpretation of diagnostic results. Their inclusion allows HEV to be examined in the context of comorbidities and specific therapies. This is highly relevant to infectious disease specialists, hepatologists, transplantation teams and internists.

The blood donor data add a public health dimension. They extend the study's significance beyond hospital diagnosis to issues of transfusion safety. The topic is particularly useful for understanding HEV as an infectious agent with multiple transmission routes.

Assessment of statistical analysis and presentation of results

The statistical section includes descriptive characteristics, intergroup comparisons and multivariable analysis. Tables reporting absolute numbers, percentages, significance levels and odds ratios allow the results to be followed systematically. Each analytical method is appropriately matched to the diverse nature of the data collected.

Scientifically, it is particularly valuable that the results of univariate comparisons are considered alongside logistic regression findings. This adds depth to the study and helps distinguish overall frequencies from specific associations identified by the regression model. The confidence intervals in Table 16 complement the p-values and facilitate assessment of estimate precision.

The dissertation also examines the age characteristics of seropositive individuals. Table 10 shows differences in mean age among the various risk groups ($F = 10.09$; $p < 0.001$), contributing to a fuller understanding of their demographic profiles.

The tables and figures organised according to the principal objectives make the material easier to understand. The combination of numerical findings and narrative interpretation provides a coherent progression from descriptive epidemiology to clinical characterisation and comparative analysis.

Extended assessment of publications and scientific communication

Four publications directly related to the dissertation are included in the submitted materials. The paper by Pishmisheva et al. in the International Journal of Current Microbiology and Applied Sciences (2020) examines HEV prevalence among haemodialysis patients in the Pazardzhik region, an important component of the epidemiological investigation.

The publication by Pishmisheva-Peleva, Kotsev et al. in Problems of Infectious and Parasitic Diseases (2024) focuses on extrahepatic manifestations in patients with acute hepatitis E in Pazardzhik during 2014–2022. This emphasis broadens the clinical perspective and considers the disease beyond typical hepatic symptoms.

The article "Hepatitis E – The Vulnerable" by Kotsev and Pishmisheva-Peleva in MedInfo (2023) is directly related to the clinical groups examined in the dissertation. The publication on HEV among individuals who consume alcohol in Preventive Medicine (2022) complements the range of risk factors considered.

The record also includes presentations at scientific forums addressing various aspects of HEV, from blood donors to clinical manifestations in the general population of the Pazardzhik region. Active presentation of these findings to colleagues demonstrates ongoing scientific communication on the subject.

Overall assessment of the contributions

The original regional dataset comprising 616 individuals makes an important contribution to Bulgarian evidence on HEV infection. Comparison of different clinical groups with individuals without risk factors provides a clear picture of the distribution of serological markers.

Another important contribution is the integration of epidemiological findings with clinical observations. Examining HEV in patients with liver disease, immunosuppression or chronic dialysis demonstrates the importance of individual clinical context. This connection between laboratory findings and clinical practice gives the dissertation genuine practical value.

The dissertation also has educational potential. Its content may be useful to medical students, infectious diseases residents and physicians involved in viral hepatitis diagnosis. The tables and comparative findings illustrate the relationships between population characteristics, laboratory markers and clinical manifestations.

Overall, the study is systematically developed and has a clearly defined scope. Its combination of original empirical data, laboratory diagnosis, statistical analysis and publications gives it a worthy place among scientific studies in infectious diseases.

Criticisms and comments: None.

Conclusion

The dissertation has a clearly defined topic and addresses an important clinical problem using original research data. The selection of patient groups, combination of methods and clinical interpretation of results form a coherent scientific work.

The study adds valuable knowledge about HEV infection in Bulgaria and is useful for diagnostic practice. I therefore give a positive assessment of the dissertation presented.

The dissertation addresses a significant problem in contemporary infectious disease pathology and presents independently collected regional data. Its principal strengths are the scale of the study, its combined methodological approach and the practical orientation of the results.

On the basis of my review of the dissertation, its abstract and the accompanying scientific materials, I give a POSITIVE scientific assessment and recommend that the esteemed Scientific Jury award Dr. Stanislav Vasilev Kotsev the educational and scientific degree of Doctor (PhD) in the doctoral programme "Infectious Diseases".

Date: 28.10.2026

Reviewer:

Assoc. Prof. Ivan Georgiev Baltadzhiev, MD, PhD