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Original article

SCREENING FOR HEPATITIS E VIRUS IN BLOOD DONORS IN NORTH MACEDONIA

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Abstract

Introduction: Hepatitis E virus (HEV) is an emerging transfusion-transmissible pathogen and a growing concern for blood safety. Since 2023, all blood donations in North Macedonia have been screened for HEV RNA, providing the first national prevalence data.

Material and methods: All blood donations collected in 2023 and 2024 were screened for HEV RNA using the Procleix UltrioPlex E nucleic acid test (NAT) on the Panther platform. Initially reactive (IR) samples were retested in duplicate, and repeatedly reactive (RR) samples underwent serological testing for anti-HEV IgM and IgG. Blood units were discarded upon the first positive NAT result. RR donors were recalled for follow-up testing after 4–6 months to assess viral clearance.

Results: A total of 107,950 donations from 71,306 donors were screened over two years. HEV RNA was initially detected in 130 samples (0.12%), with 75 confirmed as RR, yielding an overall prevalence of 0.069%. Prevalence was higher in 2024, reflecting a 64% increase. Follow-up showed viral clearance in 91.9% within 4–6 months, with all donors RNA-negative by the third follow-up. HEV positivity was not significantly associated with age or gender, though men had higher prevalence in 2023 ($p = 0.031$). Repeat donors in 2024 were more often positive than first-time donors ($p = 0.042$). Geographic clustering occurred in Skopje and Kavadarci, with seasonal peaks in March.

Conclusion: The detection of HEV among blood donors in North Macedonia highlights its relevance as a transfusion-transmissible infection and underscores the need for sustained surveillance and consideration of screening strategies.

Keywords: hepatitis E virus, blood donors, transfusion-transmissible infections, NAT, blood safety

Introduction

Hepatitis E virus (HEV) is an emerging transfusion-transmissible infection that poses a potential risk to blood safety[1]. Beyond its relevance to transfusion medicine, hepatitis E constitutes a major public health concern globally and is one of the primary causes of enterically transmitted hepatitis[2]. Several European countries have demonstrated considerable rates of HEV RNA positivity among blood donors, and universal or selective screening has been implemented to prevent transmission[3].

HEV is a small, non-enveloped, single-stranded RNA virus classified in the *Hepeviridae* family. The virus is primarily transmitted via the fecal-oral route through contaminated water and food[4,5]. Genotypes 1 and 2 cause large waterborne outbreaks in developing regions, whereas genotypes 3 and 4 are zoonotic and responsible for sporadic infections in industrialized countries[6,7].

According to the WHO, approximately 19.5 million acute HEV cases occurred worldwide in 2021, resulting in more than 3,400 deaths and highlighting its global relevance[8]. Chronic infection, mainly associated with genotype 3, is primarily observed in immunocompromised individuals[9].

HEV infection is typically characterized by an acute, self-limiting hepatitis with mild or asymptomatic presentation in most immunocompetent individuals. However, in certain high-risk groups such as pregnant women and immunocompromised patients, the infection can progress to fulminant hepatitis with a risk of severe outcomes, or may become chronic, especially with genotype 3[10,11]. Extrahepatic manifestations have also been increasingly reported, including acute pancreatitis, various neurological complications like Guillain-Barré syndrome, glomerulonephritis, thrombocytopenia, and even hemorrhagic manifestations, highlighting the systemic nature of HEV infection[12].

Screening for HEV among blood donors is an important measure to minimize the risk of transfusion-transmitted infection, as many cases remain asymptomatic and undetected. Detection relies on serological assays for anti-HEV IgM and IgG antibodies and nucleic acid testing (NAT) for HEV RNA, the most sensitive method for identifying active viremia[13]. The WHO recommends that countries evaluate local epidemiological data and transfusion-related risks when considering HEV screening implementation[14]. Since 2012, eight EU countries have introduced either universal or selective screening, reporting HEV RNA reactivity rates ranging from 1 in 744 donations in France to 1 in 8,636 in Wales, with an overall European average of 1 in 3,109 donations[15].

Global estimates indicate that nearly 939 million people, about one in eight worldwide, have been infected with HEV at some point, while between 15 and 110 million individuals are thought to have recent or ongoing infections[16]. The risk of HEV transmission through transfusion is primarily determined by the donor's viral load and the plasma volume contained in the transfused blood component[17].

Given the absence of routine screening and limited local data in some regions, there is a clear need for national prevalence studies to inform evidence-based policies that ensure transfusion safety. In North Macedonia, all blood donations are mandatorily screened for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and syphilis in accordance with national transfusion regulations[18]. A national HEV screening program has been implemented since 2023 and continues to be conducted, enabling comprehensive monitoring of HEV prevalence among blood donors.

The primary aim of this study was to determine the prevalence of HEV among blood donors in North Macedonia following the implementation of a nationwide HEV screening program. Additional objectives included:

- To assess the serological status (anti-HEV IgM and IgG) of confirmed HEV RNA-positive donors at the time of detection.
- To monitor viral clearance in repeatedly reactive donors through follow-up testing after 4–6 months.
- To analyze the prevalence of HEV positivity according to donor demographics, including age, gender, donation frequency (first-time *vs*. repeat donors), as well as geographic and seasonal distribution.

Materials and methods

This cross-sectional study was conducted at the Institute for Transfusion Medicine in Skopje and included all blood donations collected in North Macedonia between January 2023 and December 2024. Blood was obtained from voluntary, non-remunerated donors who fulfilled the national eligibility criteria for blood donation as defined by the national transfusion regulations issued by the Ministry of Health of the Republic of North Macedonia[18].

Inclusion criteria were healthy individuals of both genders, aged 18-65 years, with satisfactory medical history. Exclusion criteria included individuals with acute or chronic inflammatory or non-inflammatory diseases, malignant conditions, those previously found positive for hepatitis B, C, or HIV. From each donor, two 6 mL whole-blood samples were collected-one in a plain tube (without anticoagulant) and one in an EDTA tube.

All donations were screened for HEV RNA using nucleic acid amplification testing (NAT) with the Procleix UltrioPlex E assay on the Procleix Panther platform (Grifols Diagnostics S.A., Barcelona, Spain)[19]. UltrioPlex E assay detects 5 viruses in one reaction which enables multiplex detection of HBV DNA, HCV RNA, HIV RNA, and HEV RNA within a single test run. All testing procedures were performed strictly according to the manufacturer's instructions for use. Test results were validated and transferred from the analyzer using a laboratory information system, which enables automated data entry into the national donor information system (e-Delphyn). This system ensures centralized electronic data storage, complete traceability of donor testing results, and restricted access limited to authorized medical staff.

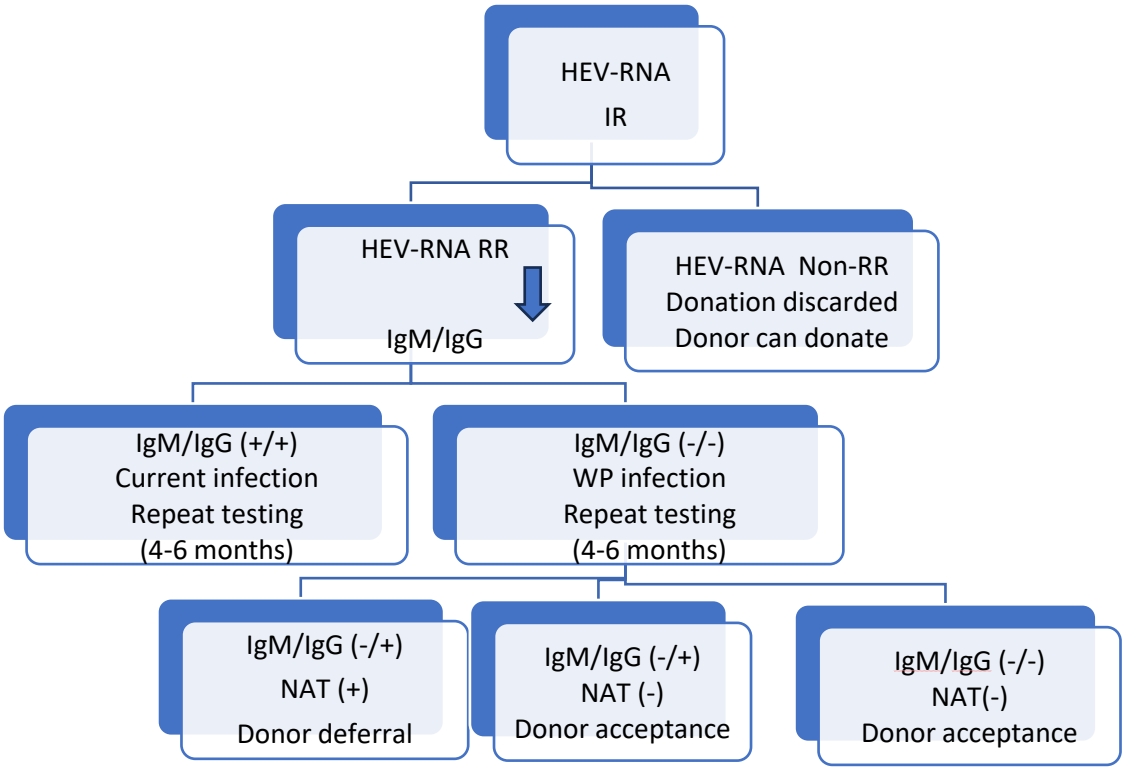
Anti-HEV IgM and IgG antibodies were determined using the VIDAS® Anti-HEV IgM and Anti-HEV IgG assays (bioMérieux, France), based on the Enzyme-Linked Fluorescent Assay (ELFA) technique, following the manufacturer's instructions[20].

Follow-up Testing of HEV RNA–Positive Donors

According to the national screening algorithm for HEV (Figure 1) applied at the Institute for Transfusion Medicine, all initially reactive (IR) HEV RNA donations were retested in duplicate using the same NAT assay to exclude false-positive results and improve reliability of the testing. This algorithm is part of the Standard Operating Procedure for transfusion-transmissible infections (TTI), testing established and approved by the Institute for Transfusion Medicine of North Macedonia and aligns with the internationally adopted blood donor testing practice and quality standards recommended by the European Directorate for the Quality of Medicines and Healthcare (EDQM)[21].

Samples with at least one repeat-reactive result were classified as repeatedly reactive (RR) and subjected to anti-HEV IgM and IgG testing. All RR donors were contacted and invited for follow-up testing 4–6 months after the initial detection to assess viral clearance or persistence and to monitor the HEV antibody status. Donors who remained HEV RNA reactive at the second testing were invited for a third follow-up 4-6 months later to monitor viral and serological dynamics. Donors could contribute multiple donations during the study period; however, all RR HEV-RNA results were from distinct individuals, and no donor was repeatedly classified as RR. Donors demonstrating viral clearance and who became HEV RNA negative 4-6 months after the last positive result and were also IgM negative were reinstated as eligible for future donation. The follow-up testing was performed using the same NAT and serological platforms as in the initial screening phase.

All blood components from IR donations, including red cells, plasma, and platelets, were automatically blocked by the donor information system and discarded to prevent any risk of transfusion-transmitted HEV infection.



*WP=window period (time between infection and the laboratory detection or to seroconversion)

Fig. 1. Testing algorithm for HEV

Statistical analysis

Data were analyzed using descriptive statistical methods. Age groups were stratified into four categories (18-30, 31-40, 41-50, and 51-65 years) to reflect the donor age distribution and to facilitate comparative analysis. Categorical variables, including gender, age group, donation frequency, geographic origin, and seasonal distribution, were summarized as absolute numbers and percentages. Continuous variables, such as donor age, were expressed as mean ± standard deviation (SD) and compared between groups using the independent Student's *t*-test.

Associations between HEV positivity and demographic donor characteristics were examined using the Chi-square (χ^2) test and Fisher's exact test. Statistical significance was set at $p < 0.05$.

Results

During the two-year screening period (2023-2024), a total of 107,950 blood donations from 71,306 individual donors were tested for HEV RNA. In total, 130 donations were IR, of which 75(57.7%) were subsequently confirmed as RR. The overall prevalence of RR HEV RNA-positive donations was 0.069%. In 2023, 30 (0.053%) positive cases were detected among 56,450 donations, while in 2024, 45 (0.087%) positive cases were identified among 51,500 donations (Table 1).

Table 1. Annual distribution of blood donations, donors, and HEV NAT-RR prevalence

Year	Donations n (%)	Blood donors n (%)	NAT-HEV RR donations n (%)
2023	56450(52.29)	36670(64.96)	30(0.053)
2024	51500(47.71)	34636(67.25)	45(0.087)
Total	107950(100)	71306(100)	75(0.069)

It should be noted that donations and donors are distinct terms, as a single donor may contribute more than one donation within the analyzed timeframe.

For comparison with other TTI in 2023, the prevalence of HBV, HCV, and HIV among blood donations was 0.239%, 0.008%, and 0.005%, respectively. In 2024, the corresponding prevalences were 0.167%, 0.014%, and 0.012%. HEV RNA prevalence (0.053% in 2023 and 0.087% in 2024) exceeded that of HCV and HIV in both years.

Among the 75 HEV-RNA RR positive donors, 55(73.3%) showed no serological response in the initial sample (IgM-/IgG-), whereas 20(26.7%) were IgM+/IgG+ (Figure 2).

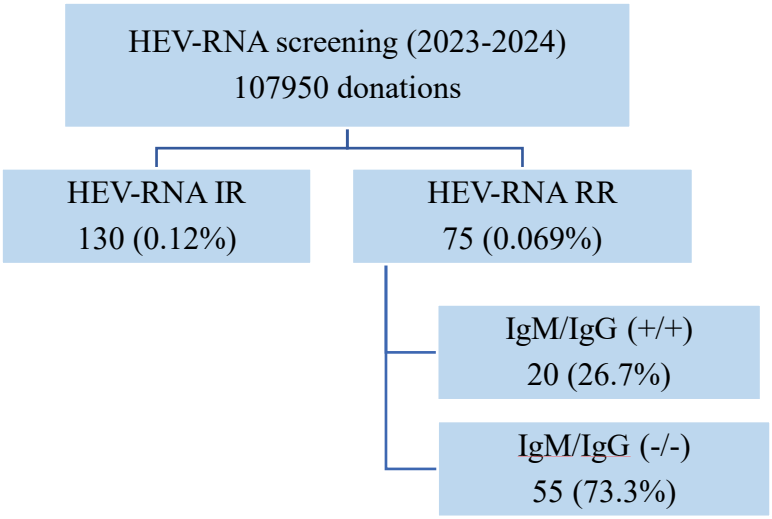


Fig. 2. HEV-RNA screening of blood donations and serological distribution of HEV-RNA repeatedly reactive donors

A second sample, collected 4-6 months later from 62 donors, revealed that only 5(8.1%) remained HEV-RNA positive with IgM+/IgG+, while 57(91.9%) had cleared the virus (HEV-RNA). Among these HEV-RNA-negative donors, 7(12.3%) were IgM+/IgG+, and 50 (87.7%) were IgM−/IgG+. A third follow-up sample, obtained from 10 donors, showed that all were HEV-RNA negative. Of these, 1(10%) remained IgM+/IgG+, whereas 9(90%) were IgM−/IgG+ (Figure 3).

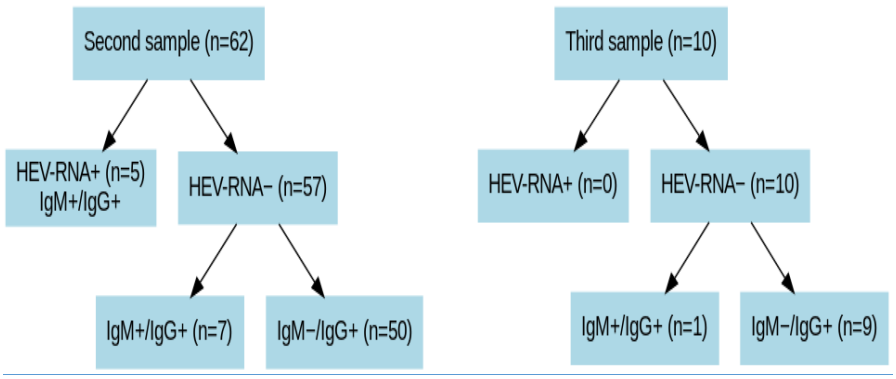


Fig. 3. HEV RNA status and serological dynamics during follow-up testing of HEV-RNA repeatedly reactive blood donors

Gender distribution during the 2023/24 period showed that among 57,695(80.91%) male donors, 65(0.11%) were HEV positive, while among 13,611(19.09%) female donors, 10 (0.07%) tested positive. The difference in the distribution of HEV-positive and HEV-negative donors between males and females was statistically non-significant (p=0.2) (Table 2).

Table 2. Distribution of HEV-positive and HEV-negative donors by gender

Gender	2023 - 2024	HEV		p-value
	n (%)	positive donors n (%)	negative donors n (%)	
Male	57695 (80.91)	65(0.11)	57630(99.89)	X^2=1.6
Female	13611 (19.09)	10(0.07)	13601(99.93)	1 p=0.2
Total	71306 (100)	75(0.11)	71231(99.89)	

X^2(Chi-square)

In 2023, gender distribution analysis revealed that 29 (0.097%) male donors tested HEV positive in comparison with 1(0.015%) female donor. The higher prevalence of HEV positivity among male donors compared to female donors in this year was statistically significant (p=0.031). In 2024, donor gender showed no significant association with HEV positivity (p=0.93), and the prevalence of HEV-positive donors was identical in both genders (0.13%).

The age of HEV-positive donors in 2023 ranged from 20 to 63 years, while in 2024 it ranged from 18 to 59 years. The mean age of HEV-positive donors in 2023 was 41.9 ± 12.2 years, and they were insignificantly older than HEV-positive donors in 2024, whose mean age was 37.7 ± 10.8 years (p=0.126) (Table 3).

Table 3. Mean age of HEV-positive blood donors

Year	Age / years			p-level
	n (%)	mean ± SD	min- max	
2023	30 (40)	41.9 ± 12.2	20 – 63	t=1.55
2024	45 (60)	37.7 ± 10.8	18 – 59	p=0.126

t(Student t-test)

The age distribution of donors during the analyzed two-year period (2023/2024) showed 18 (0.09%) HEV-positive donors in the 18-30 age group, 25(0.13%) in the 31-40 age group, 18 (0.09%) in the 41-50 age group, and 14(0.12%) in the 51-65 age group (Table 4). The tested difference in the age distribution of HEV-positive and HEV-negative donors was not statistically significant (p=0.57). The higher prevalence of HEV-positive donors in the 31-40 age group compared with the other age groups was not sufficient to reach statistical significance.

Table 4. Age distribution of HEV-positive and HEV-negative donors

Age (years)	2023 - 2024	HEV		p-value
	n (%)	Positive donors n (%)	Negative donors n (%)	
18-30	19161 (26.87)	18 (0.09)	19143 (99.91)	X^2=2.0 p=0.57
31-40	19536 (27.4)	25 (0.13)	19511 (99.87)	
41-50	20660 (28.97)	18 (0.09)	20642 (99.91)	
51-65	11949 (16.76)	14 (0.12)	11935 (99.88)	
Total	71306 (100)	75 (0.11)	71231(99.89)	

X^2(Chi-square)

During the 2023-2024 period, repeat donors were insignificantly more frequently HEV positive compared to first-time donors, 64 (0.12%) *vs*. 11 (0.06%), $p = 0.063$ (Table 5). A statistically significant difference between HEV-positive and HEV-negative donors in relation to blood donation frequency was observed in 2024 ($p = 0.042$). Repeat donors were significantly more often HEV- positive than first-time donors (0.15% *vs*. 0.065%).

Table 5. Distribution of HEV-positive and HEV-negative donors by donation frequency

Donation frequency	2023 - 2024 HEV n (%)	Positive donors n (%)	Negative donors n (%)	p-value
First- time donors	16974 (23.8)	11 (0.06)	16963 (99.94)	X^2=3.46 p=0.063
Repeat donors	54332 (76.2)	64 (0.12)	54268 (99.88)	
Total	71306 (100)	75 (0.11)	71231 (99.89)	

X^2(Chi-square)

The analysis of geographic distribution revealed that the highest proportion of HEV-positive donors originated from Skopje 18 (24%), followed by Kavadarci 14 (18.67%) donors, whereas the remaining regions accounted for smaller shares.

According to the seasonal distribution presented in Table 6, the highest number of HEV-positive donors was registered in March, 15 (20%) cases. By year, in 2023 the peak was observed in February with 5 (16.67%) cases, while in 2024 the highest number was recorded in March with 11(24.44%) cases.

Table 6. Seasonal distribution of HEV positive donors

Month	n (%)	Year 2023 n (%)	2024 n (%)
January	8(10.67)	2(6.67)	6(13.33)
February	10(13.33)	5(16.67)	5(11.11)
March	15(20)	4(13.33)	11(24.44)
April	8(10.67)	2(6.67)	6(13.33)
May	3(4)	2(6.67)	1(2.22)
June	6(8)	2(6.67)	4(8.89)
July	4(5.33)	1(3.33)	3(6.67)
August	2(2.37)	1(3.33)	1(2.22)
September	3(4)	2(6.67)	1(2.22)
October	5(6.67)	2(6.67)	3(6.67)
November	5(6.67)	2(6.67)	3(6.67)
December	6(8)	5(16.67)	1(2.22)
Total	75	30(40)	45(60)

Discussion

The present study offers comprehensive data on HEV RNA prevalence among blood donors in North Macedonia during 2023–2024. The overall prevalence of HEV RNA-positive donations was 0.069%, aligning with European data where prevalence ranges approximately from 0.01% to 0.13%[15].

With regard to donations and donors, our study calculated an HEV incidence of 1:1,439 in relation to total blood donations, and 1:951 in relation to total donors over a two-year period. These values are comparable to those reported in a German study[22], which found an incidence of 1:1,474 in donations and 1:241 in donors over an eight-year observation period. Findings from the German study indicated a notable prevalence of HEV, leading the authors to stress the necessity of HEV RNA screening in blood donation practice. In comparison, our results demonstrate a comparable prevalence despite being based on a much shorter, two-year period.

The 64% increase in HEV prevalence in 2024 (0.087% *vs*. 0.053%) indicates temporal fluctuation, underscoring the need for continuous monitoring to assess its impact on blood safety. This finding strongly supports the consideration of implementing routine HEV RNA screening in blood donors in North Macedonia, in line with recommendations already adopted in several European countries.

The higher prevalence of HEV RNA compared to other TTI underscores its growing epidemiological relevance. Although the overall prevalence rates remain low, HEV was detected more frequently than HCV and HIV, despite these viruses being part of the mandatory screening panel. These findings emphasize that HEV represents a relevant transfusion-transmissible risk, exceeding viruses that are already included in mandatory donor screening.

Several recent studies have demonstrated that HEV infection remains a significant transfusion-transmitted risk in many regions, including industrialized countries where zoonotic and foodborne transmission routes predominate. In Catalonia, Spain, universal screening of blood donors between 2017 and 2020 identified 1 HEV RNA-positive donation per 4,341 donations[23]. Similarly, data from England demonstrated that after the transition from selective to universal HEV RNA screening, 480 donations out of 1,838,747 tested were confirmed RNA-positive[24]. A study conducted in Bavaria, Germany, demonstrated that HEV infection remains an important public health concern, with a noticeable increase in reported cases and significant regional differences in incidence rates, indicating ongoing zoonotic transmission through contaminated food products and close contact with animal reservoirs[25]. Hogema *et al.* concluded that the incidence of HEV infection among Dutch blood donors was relatively high and showed an increasing trend during the study period, with HEV RNA detected in approximately 1 in every 762 donations used for solvent/detergent-treated plasma production[26].

Japan was among the earliest to introduce nationwide HEV NAT screening, and evidence from this setting demonstrated that transfusion-transmitted HEV infections are possible, underlining the importance of routine testing to ensure blood safety[27]. In Eastern Europe, a recent study from Russia showed that the average HEV RNA prevalence among voluntary blood donors was 0.024%, equivalent to 1 case per 4,081 donations[28].

Follow-up testing revealed that most HEV-positive donors cleared the viremia within 4–6 months, and all donors with a third follow-up sample were RNA negative, which is typical for the self-limiting course of infection in immunocompetent individuals. This observation is in line with prospective serial analyses in asymptomatic German blood donors, where viremia and seroconversion were shown to be transient and usually resolved with the development of IgG antibodies[29].

The high proportion of seronegative (IgM-/IgG-) donors at the time of donation in our study matches reports from other cohorts, where a significant number of viremic donors were within the "window period." This highlights the limitations of serology alone and underscores the advantage of NAT screening in detecting early infections[30] .

At the second follow-up (4-6 months after the initial donation), most donors had cleared HEV RNA, confirming the transient nature of viremia. Only a small proportion (n=5; 8.1%) remained HEV-RNA positive, while 12(19.4%) showed persistent IgM reactivity, indicating ongoing or recent immune response. By the third follow-up, all donors were HEV-RNA negative and IgG positive, consistent with complete viral clearance and seroconversion. Among donors who were initially IgM+/IgG+, all subsequently cleared viremia during follow-up. Only one donor remained IgM+/IgG+ at the third sampling despite being HEV-RNA negative. This donor was referred for infectious disease consultation and follow-up, as persistent IgM positivity may reflect delayed antibody decline rather than ongoing infection. Overall, these findings demonstrate that HEV infection in blood donors follows a predictable, self-limiting course with stable antibody dynamics over time.

The gender analysis showed no significant difference over the two-year period, though 2023 exhibited higher HEV positivity among men, a finding mirrored in other European datasets where male donors sometimes show elevated HEV infection rates[31].

The age distribution of HEV-positive donors in our study demonstrated that infections occurred across all age groups, without statistically significant differences. These findings suggest that, within the donor population, HEV infection is not strongly associated with age, which is consistent with reports from other European cohorts[22] where seroprevalence and viremia were broadly distributed across adult age groups. The absence of an age-specific pattern may reflect the widespread exposure routes of HEV in the general population rather than age-dependent risk factors.

HEV RNA positivity was slightly higher among repeat donors, contrasting with the conventional assumption that first-time donors represent a higher-risk group. Although this difference reached statistical significance only in 2024, similar findings have been reported in other settings. Tedder *et al*.[32] analyzed the virology, serology, and demographics of HEV-viremic blood donors in the United Kingdom and found that a significantly greater proportion of HEV-infected donations originated from repeat donors rather than first-time donors. This highlights that repeated donation does not eliminate the risk of HEV viremia and that cumulative environmental or dietary exposures may contribute to the higher prevalence observed among repeat donors.

Geographically, HEV-positive donors clustered in Skopje and Kavadarci, suggesting possible localized epidemiologic hotspots. Such clustering may reflect prevalent dietary or zoonotic transmission routes, particularly through pork or game meat consumption, as supported by recent European findings. In the Netherlands, Slot *et al.* reported that meat-eating donors had significantly higher anti-HEV IgG seroprevalence than vegetarian donors (20.5% *vs*. 12.4%, p=0.002), reinforcing the role of meat consumption as a key risk factor for HEV infection[33].

In our cohort, seasonal analysis showed a peak of HEV-positive donors in March, with year-to-year variability (February in 2023 *vs*. March in 2024). By contrast, German blood donor surveillance demonstrated the highest HEV RNA incidence in the summer months of June and July, with rates around 0.084-0.083%[22]. This discrepancy highlights the regional heterogeneity of HEV seasonality, suggesting that local environmental and dietary factors strongly influence temporal patterns of infection.

Taken together, our results demonstrate that HEV infection among blood donors in North Macedonia mirrors trends reported across Europe, with low but non-negligible prevalence, transient viremia, and evidence of regional and seasonal variation. Importantly, the detection of viremic but seronegative donors emphasizes the limitations of serology and the critical role of NAT in ensuring transfusion safety[1]. Screening blood donations for HEV is especially important for immunosuppressed and transplant recipients, who face the highest risk of severe or even fatal outcomes. However, it should also be considered for immunocompetent patients, as transfusion-transmitted HEV can occasionally cause acute illness or serious complications under certain clinical circumstances[34].

Conclusion

This study confirms the presence of HEV among healthy blood donors in North Macedonia, with prevalence levels comparable to those observed in other European countries. While most infections were transient and self-limiting, the presence of asymptomatic RNA-positive donors poses a transfusion risk, especially for immunosuppressed and transplant recipients. HEV prevalence exceeded that of HCV and HIV during the same period, highlighting its relative significance among transfusion-transmissible infections. Continuous surveillance and consideration of routine HEV screening are essential to further improve blood safety.

Conflict of interest statement. None declared.

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