Received: 26 January 2022 | Accepted: 11 April 2022

DOI: 10.1111/jvh.13682

JOURNAL OF VIRAL HEPATITIS JVH WILEY

SHORT COMMUNICATION

Evaluating the prevalence of Hepatitis E virus infection in a large cohort of European blood donors, 2015–2018

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Abstract

Hepatitis E virus (HEV) is endemic in Europe. However, standardized methods for the surveillance of HEV viremia in the general population are lacking. This study aimed to compare the incidence of HEV among blood donors in two European countries, Germany and Portugal, during the period 2015-2018. The seasonal distribution of HEV infection, as well as host risk factors including age, sex, and blood group phenotype were explored. A total of 191,236 donations from Germany and Portugal were tested for HEV RNA in plasma mini-pools of up to 96 donations using an internally controlled reverse transcription real-time PCR (RT-PCR) assay. The 95% cut-off of the assay was 15 International Units (IU)/mL (CI 10-35 IU/mL) as determined by dilution of the WHO International Standard for HEV RNA. Blood type was determined by agglutination and pattern recognition using the Beckmann Coulter PK 7300 AB0- and Rhesus-Assay. The overall positivity rate was 0.09% with significantly more infections observed in the German cohort ($p < 0.0001$). Infections peaked in the summer months, and investigation of risk factors revealed that incidence was significantly higher amongst males ($p = 0.0002$), but was not associated with ABO or Rh(D) blood group phenotypes. No significant relationships between risk factors and viral load were observed. Our findings confirm that HEV infections are highly prevalent in Europe, even amongst otherwise healthy blood donors. Increasing awareness of the seasonal spread and risk factors for HEV transmission is of great importance for individuals susceptible to more severe forms of the disease, such as immunocompromised patients.

KEYWORDS
ABO, hepatitis E virus, rhesus, RT-PCR, transfusion medicine, viremia

Abbreviations: CI, confidence interval; CP value, crossing point value; ECDC, European Centre for Disease Prevention and Control; EU/EEA, European Union/European Economic Area; HEV, Hepatitis E virus; IU, international units; OR, odds ratio; RT-PCR, real-time polymerase chain reaction.

1 | INTRODUCTION

Hepatitis E virus (HEV) is a small RNA virus and the leading cause of acute viral hepatitis in humans. In Europe, most HEV cases are caused by genotype 3 and transmitted by improperly cooked meat, direct contact with infected animals and contaminated blood products. Annually, at least 2 million locally acquired HEV infections occur in Europe. Acute HEV infection is typically self-limiting and sub-clinical.[1] However, in immunocompromised patients, the virus can persist and cause chronic disease.[2] Chronic HEV is associated with more severe liver complications such as fulminant hepatitis and extrahepatic manifestations,[1] making HEV a significant public health risk amongst vulnerable groups (Table 1).

There is currently no mandate for reporting HEV infection (acute or chronic) at EU/EEA level. Instead, transmission surveillance relies only at national level.[3] Although the frequency of HEV in European blood donors is higher than previously thought, not all EU countries test for HEV in blood transfusion products.[1,3] Furthermore, many prevalence studies focus on serological data of previous exposure instead of incidence of active infection. The risk of severe disease in immunocompromised individuals,[2] coupled with the lack of standardized international screening methods for HEV warrants further investigation of HEV epidemiology and risk factors for infection.

In addition to environmental risks, host factors may also play a role in HEV susceptibility. Blood antigens are known to act as receptors for host immune and inflammatory responses and can mediate innate responses to invading pathogens.[4] The ABO erythrocyte antigen system and Rh(D) phenotype play important roles in clinical practice. Associations between ABO blood group and susceptibility to infection and infection-associated disease severity have been described for other liver pathogens such as hepatitis B virus.[4]

In this study, we investigated the incidence of acute HEV infection in blood donors in two European countries, Germany and Portugal, during the period 2015–2018. Annual and seasonal infection dynamics and host risk factors including age, sex and blood group were also assessed.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

All samples included in the study were pseudonymized and no identity key was available. Therefore, as the original donor identities could not be backtracked, no ethical permit was required according to the Regional Ethical Review Board in Stockholm and the ethical standards of the Declaration of Helsinki.

2.2 | Screening and blood group typing of plasma donations

Plasma donations from German (n = 170,843) and Portuguese (n = 20,393) donors were analysed for the presence of HEV RNA. German donors where blood phenotype data was not available (n = 3720) or with an undetermined result (n = 1) were removed. Screening was performed in plasma mini-pools of up to 96 donations using a proprietary internally controlled real-time reverse transcription polymerase chain reaction (RT-PCR) assay with 95% cut-off at 15 International Units (IU)/mL determined using the WHO International Standard for HEV RNA. The assay is validated to detect the HEV geno- and subtypes 1a, 1e, 2, 3a, 3b, 3c, 3e, 3f, 3, 4c and 4 g. Samples with a crossing point (Cp) value determined by the PCR software, or with a visible amplification curve, were considered HEV positive. These donations were excluded from downstream pharmaceutical production. Cp value was defined as the PCR cycle where the tangent of the amplification curve had the greatest slope. Blood type was determined by agglutination and pattern recognition using the Beckmann Coulter PK 7300 AB0- and Rhesus-Assay. All HEV+ cases were confirmed negative for co-infection with HIV and hepatitis A, B and C viruses using proprietary internally controlled RT-PCR assays of mini-pools of up to 480 donations with 95% cut-off levels of 12, 26, 31 or 9.4 IU/ml respectively.

TABLE 1 Summary demographics for HEV-screened plasma donors stratified by blood type

	Total	A	AB	B	O	Rh(D) neg	Rh(D) pos
Germany							
N	167,122	67,736	27,364	17,054	54,968	28,950	138,172
Age (IQR)	34 (25–49)	35 (26–50)	35 (26–50)	32 (24–48)	32 (25–48)	35 (26–49)	34 (25–49)
Male (%)	98,738 (59.08%)	40,537 (59.9%)	16,074 (58.74%)	9975 (58.49%)	32,152 (58.49%)	16,503 (57.01%)	82,235 (59.51%)
HEV+ (%)	167 (0.100%)	63 (0.093%)	36 (0.132%)	18 (0.106%)	50 (0.091%)	31 (0.107%)	136 (0.098%)
Portugal							
N	20,393	–	–	–	–	–	–
HEV+ (%)	4 (0.020%)	–	–	–	–	–	–

Note: *N* is the number of plasma donations that were screened for HEV RNA between the period 2015–2018. Blood group percents are relative to all individuals in the German cohort.

Abbreviations: D antigen; HEV, hepatitis E virus; Rh(D), Rh blood group.

2.3 | Statistical analysis

All datasets initially underwent normality testing. Categorical and numerical comparisons were performed using GraphPad Prism (v9.0). Multivariate logistic regression analysis was performed in the statistical software R (v4.0.5.).

3 | RESULTS

3.1 | HEV incidence in Germany and Portugal cohorts

Of 187,515 donations analysed between 2015 and 2018, 171 tested positive for HEV RNA, resulting in an overall HEV positivity rate of 0.09%. The frequency of HEV infection during the screening period in the German cohort (HEV+ $n = 167$; Total $n = 167{,}122$) was five times higher than the Portuguese cohort (HEV+ $n = 4$; Total $n = 20{,}393$) (0.10% vs. 0.02%, respectively) (Figure 1A) ($p < 0.0001$; Fisher's exact test). The incidence rates varied with year showing fluctuations in HEV+ donations over time. Age, sex and blood typing data were available for the German cohort hence all subsequent analyses were performed on Germany only.

3.2 | Monthly infection rates

We hypothesized that human behavioural changes in the summer months may influence HEV transmission. Indeed, the highest incidence of HEV infection was observed during May–July (Figure 1B).

3.3 | Age, sex, blood groups and HEV incidence

Although HEV RNA was detected in all age groups, we observed the highest incidence of infection amongst the 41–50 years age group (Figure 1C).

We evaluated the categorical variables sex, Rh(D) phenotype and ABO blood group as risk factors for HEV infection by multivariate logistic regression analysis (Figure 1D). The only variable associated with risk for HEV infection was sex, where males had a higher risk compared with females (OR 1.88, $p = 0.0003$).

3.4 | HEV viremia and host factors

We also investigated the role of host factors on plasma HEV RNA levels. By assessing the HEV RT-PCR Cp values which inversely correlate with the viral load, we found that the viral load was not impacted by age (rho = −0.128, $p = 0.0992$; Spearman correlation analysis) (Figure S1A), sex ($p = 0.7566$; Mann–Whitney U test) (Figure S1B), or ABO blood group ($p = 0.1565$; Kruskal–Wallis test) and rhesus factor ($p = 0.2746$; Mann–Whitney U test) (Figure S1C).

4 | DISCUSSION

The present study investigated HEV infection in one of the largest European cohorts of blood donors spanning from 2015 to 2018. Although antibody prevalence rate was reported from a nation-wide survey in Portugal in 2018,[5] this study highlights the prevalence of active HEV infection on large-scale population level in a consecutive four-year study period. We found that HEV incidence in Portugal was significantly lower than in Germany, confirming blood transfusion as a relevant infection risk in Germany, as previously described.[2] According to the European Centre for Disease Prevention and Control, European HEV cases have been steadily increasing in the period 2005–2015. Interestingly, we observed fluctuations in the annual incidence of HEV infection in this cohort, in line with a similar study in England from the same time period.[6]

Consumption of undercooked pork products is the primary risk factor for acquiring HEV in Germany.[1,2] Therefore, we assumed that HEV incidence rates might differ in summer, when cooking habits and behaviours may change. We found that HEV infections were most prevalent in May–July, confirming a seasonal distribution.

HEV infection amongst German adults is known to increase with age, before levelling-off at 60 years old.[7] This was confirmed in our cohort with an increase in incidence by age which peaked amongst the 41–50 years age group, and sharply declined after 60+. It is unclear why the HEV acquisition risk is lower in the older population but may reflect varying dietary habits and cooking practices in different age groups.

Whereas no significant difference in HEV seroconversion between the sexes was seen in another German cohort,[7] we observed significantly higher incidences of HEV infections in males, as previously described.[2] It is unclear whether it is due to male-specific susceptibility or other risk factors such as dietary habits and underlying medical conditions. For example, a report from 2015 showed that men ate almost twice as much meat and processed meat products than women, which would increase their HEV exposure risk.[8]

Although no significant difference in ABO and Rh(D) blood group distribution was observed in the HEV- vs. HEV+ donors, we cannot conclude that blood groups do not play a role in HEV pathogenesis. While we demonstrated that blood groups are not associated with susceptibility to HEV, they may play a role in disease severity and persistence, factors outside the scope of this study. Given that over 90% of HEV cases are expected asymptomatic, longitudinal studies of this disease are challenging at population level. However, follow-up studies in those with symptomatic and/or severe HEV infection may provide information on the role of blood groups in clinical outcomes.

The strengths of this study were the large sample size and standardized detection method which allowed direct comparison of HEV positivity between the two study cohorts. Serological analysis for HEV-specific antibodies would have added value to this study, but the purpose here was to assess the risk for active infection, that is, HEV RNA in blood. Another limitation was the lack of regional data. Since many HEV outbreaks are local,[6] it would have been interesting

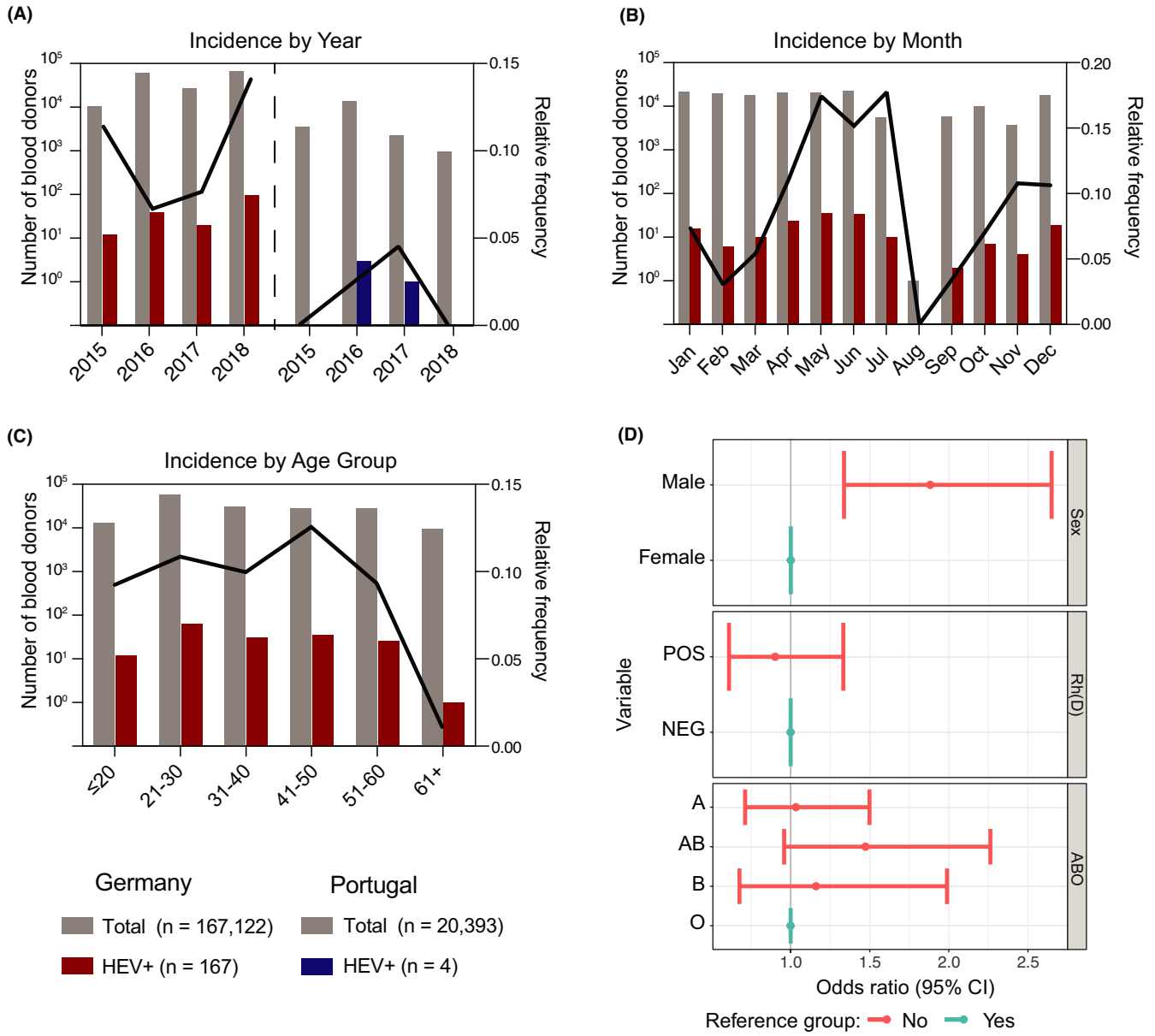

FIGURE 1 Temporal and risk factors associated with HEV infection in blood donors. HEV RNA positive plasma donations were plotted relative to the total cohort (black line = relative frequency) by (A) donation site/year, (B) month, and (C) age. (D) Logistic regression analysis showed that sex was the only significant genetic risk factor included in the study (***$p = 0.0002$)

to correlate the present findings to regional data such as rural or urban backgrounds.

In summary, the data in the present study reveals that the HEV RNA prevalence in blood donors differs between European countries. Increasing awareness of HEV risk factors in endemic sites or countries should be encouraged. Our results also suggest that screening of blood products for HEV should be mandated in Europe, particularly for use in immunocompromised vulnerable patient groups.

AUTHORS CONTRIBUTIONS

RG conceived the study design. UF, ME and HH collected the data. KH, LA, and RG analysed the data. RG, KH, and MSC interpreted the data. RG supervised the work. RG and KH wrote the manuscript. All authors reviewed and revised the manuscript critically.

ACKNOWLEDGEMENT

This study was made possible owing to a Karolinska Institutet Careers Service Internship (Katie Healy) at Octapharma AB. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

UF, ME, LB, HH and RG are all employed at Octapharma, a company specialized in the development and production of human proteins from plasma and cell lines. The other authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Healy K, Freij U, Ellerstad M, et al. Evaluating the prevalence of Hepatitis E virus infection in a large cohort of European blood donors, 2015–2018. *J Viral Hepat*. 2022;29:835-839. doi: 10.1111/jvh.13682