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Hepatitis E virus prevalence in Flemish blood donors

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Abstract

Transmission of hepatitis E virus (HEV) through transfusion of blood components has already been reported in several European countries. Here, we assessed the HEV prevalence in Flemish blood donors. This study is of importance in order to assess the risk of HEV transmission through blood transfusion. We analysed 38 137 blood donation samples that were collected by the Red Cross Flanders during the period May-June 2015. All samples were screened for the presence of HEV RNA and a selection for HEV-specific IgM/IgG. After pooling per 6, 11 pools reacted positive during RNA screening. Reactive pools were deconstructed, and individual samples were retested. After deconstruction, seven samples were confirmed as HEV RNA positive. Serological screening of the confirmed RNA-positive samples showed that six out of these seven samples were HEV IgM positive, of which three donors were also IgG positive. Serological screening was also performed on the samples that constituted the four initially HEV RNA reactive pools where RNA positivity was not confirmed on the individual level. In three pools, we found indirect evidence of recent HEV exposure. Within 356 randomly selected samples, 31 donations were HEV IgG positive. Here we show that at least 1:5448 of blood donations in Flanders may originate from donors that are actively infected with HEV. Upon transfusion, these donations may pose a major threat towards patients at risk. Finally, a serological analysis showed that the anti-HEV IgG prevalence in Flemish blood donors is 8.71%.

KEYWORDS
blood donors, hepatitis E virus, transmission

1 | INTRODUCTION

Hepatitis E virus (HEV) is a worldwide underdiagnosed virus responsible for at least 20 million infections yearly.[1,2] Although HEV is mainly transmitted by faecal-oral route, rising numbers of transmissions through blood transfusion are documented worldwide. Since HEV-infected adults are commonly asymptomatic when viremic, they would usually qualify as blood donors. Therefore, a hepatitis E infection is considered as an emerging concern in the context of blood transfusion.[3] Documenting the transmission of HEV to blood recipients and estimating the risk of transfusion-transmitted HEV has been challenging, despite the relatively high seroprevalence and identification of HEV RNA in qualified donors. This is due to the difficulty of linking donors to recipients, the long incubation period of 30 days or more, the absence of overt clinical symptoms and the predominant risk of food-borne transmission. Despite these challenges, numerous studies have described transmission through blood transfusion.[3] Nonetheless, no transmission via plasma-derived medicinal products (PDMPs) obtained after virus inactivation and/or removal

Abbreviations: CI, confidence interval; Ct, PCR threshold cycle; HEV, hepatitis E virus; LOD, limit of detection; NA, not available; ND, not detected; NI, sample not identified; OD/CO, optical density/cut-off; PASs, platelet additive solutions; PDMPs, plasma-derived medicinal products; PI, pathogen inactivation; TTIDs, transfusion-transmitted infectious diseases.

steps has been documented so far.[4] Since no transfusion transmission data from Belgium is available, we assessed the HEV prevalence in Flemish blood donations. It is of importance to determine the risk of HEV transmission through blood transfusion in our region, especially for patients at high risk for complications.

2 | MATERIALS AND METHODS

2.1 | Sample collection

The 38 137 analysed EDTA-plasma samples from individual blood donations were collected at the Red Cross Flanders during the period May to June 2015. The samples were stored at −20°C until they were analysed from June until September 2017. All donations originated from consenting donors. In order to preserve privacy, only gender and age of the donors were known. Postal codes were retrospectively obtained in a blinded manner for the HEV RNA-positive donations. This study was approved by the ethical committee of the Ghent University Hospital (registration number: B670201423079).

2.2 | HEV RNA detection

Previously frozen samples were initially pooled per 6 and screened for the presence of HEV RNA using the cobas® HEV test on the cobas® 6800 System (Roche Diagnostics GmBH). The cobas® HEV assay is a real-time PCR test for the detection of hepatitis E virus RNA (genotypes 1-4) in human plasma. The assay was carried out according to the manufacturer's instructions, except that the samples were diluted with PBS when screened on individual level and the storage period that was longer than the 12 months prior to testing. The assay has a 95% limit of detection (LOD) of 18.6 IU/mL. In a second phase, RNA reactive pools were deconstructed and analysed individually using the same methodology. Because of volume limitations, individual samples were diluted with an equal volume of PBS. The sample ID reported in this manuscript is constituted of the pool ID followed by the number of the individual samples in the pool.

An estimation of the HEV viral load in positive samples was possible by inclusion of a dilution series (range 10^2-10^5 IU/mL) of the WHO International Standard containing a genotype 3a HEV strain (Paul-Ehrlich-Institut).

2.3 | HEV serology

HEV antibody was detected with the Wantai IgM and IgG detection assays (Beijing Wantai Biological Pharmacy Enterprise Co.) in accordance with the manufacturer's instructions. The presence of HEV IgG and IgM was determined in all individual samples originating from HEV RNA-positive pools. Additionally, a selection of 356 randomly chosen samples that scored HEV RNA negative when tested in pool format was screened for the presence of HEV IgG. HEV-specific IgM was determined in the IgG-positive/RNA-negative samples.

2.4 | Statistics

Power calculations of the study were made using the Sas Power and Sample Size program (SAS). The analysis for the required number of random samples to determine the anti-HEV IgG prevalence was based on the exact test for binomial proportion. The power of our study was calculated based on a null proportion of 0%, a binominal proportion of 14% and a nominal alpha of 0.05. An a priori calculation showed that a sample size of n = 356 (number of random samples) would result in a power >0.999. A 95% Wilson score confidence interval (CI) for proportions was calculated using the program R, version 3.3.2 (The R foundation for Statistical Computing).

The correlation between anti-HEV IgG prevalence on one hand and age and gender on the other hand was calculated using the chi-square test using the GraphPad program (GraphPad Software). A 95% Wilson score confidence interval (CI) for differences in proportions was calculated using the program R.

3 | RESULTS

3.1 | HEV RNA prevalence in Flemish blood donations

After pooling of the 38.137 blood donations (6.396 pools), the presence of HEV RNA was determined in each pool. During initial HEV RNA screening, 11 pools reacted positive. After deconstruction of the RNA reactive pools, seven individual blood donations (7/38 137; 0.018%) originating from seven distinct pools were confirmed as HEV RNA positive (range 1.53×10^2-8.71×10^3 IU/mL; Table 1). From here on, these seven RNA-positive samples will be referred to as RNA-confirmed samples. Samples constituting the four pools where no RNA-positive sample was found after deconstruction will be referred to as RNA-unconfirmed samples. Geographically, two of the RNA-positive donors are living in the province East-Flanders, two in West-Flanders, one in Limburg, one in Brussels and one living abroad in the Netherlands.

3.2 | Signs of past HEV infection in Flemish blood donations

In a next step, we have performed a serological screening on all individual samples from the 11 pools that tested positive during the first HEV RNA screening. These pools include the seven reactive pools each containing one confirmed RNA-positive sample after deconstruction and the four reactive pools where no RNA-positive sample could be identified after deconstruction (Figure 1). Serological screening of the RNA-confirmed samples showed that six out of seven samples were HEV IgM positive, of which three donors were also IgG positive. The remaining 34 samples constituting these seven reactive pools were HEV antibody negative (data not shown). Serological screening was also performed on the four RNA-unconfirmed pools. One pool contained a sample that was IgM and IgG positive, suggesting recent exposure. Two pools comprised a single sample being IgG

TABLE 1 Overview of HEV RNA levels in RT-qPCR reactive pools

Pool ID	Ct pool	Ct individual sample	HEV RN (IU/mL)
6417	40.95	NI	NI
401	37.99	37.98	9.48×10^2
2595	44.98	NI	NI
2645	34.52	34.28	8.71×10^3
5397	35.53	34.93	5.90×10^3
5642	40.03	38.80	5.80×10^2
4395	41.47	NI	NI
5740	40.33	41.03	1.53×10^2
6013	40.93	40.83	1.72×10^2
1096	38.43	38.35	7.60×10^2
6228	41.80	NI	NI

Abbreviations: Ct, PCR threshold cycle; NI, sample not identified.

positive, demonstrating past infection. Finally, the fourth pool did not include a sample with positive serology (Figure 1; Table 2).

In addition, 356 samples that scored HEV RNA negative when screened on pool level were randomly selected for serological screening, in order to verify the presence of anti-HEV antibodies in samples that are RNA negative. From the 356 individual samples tested, 31 donations were anti-HEV IgG positive, resulting in an IgG seroprevalence of 8.71% [6.20%-12.10%] in Flemish blood donors (Figure 1).

We then compared age and gender data from the random samples in order to reveal possible epidemiological trends. The 356 donors were comprised of 196 (55.06%) male and 160 (44.94%) female donors. Of the 31 IgG-positive donors, 20 were male (64.52%) and 11 were female (35.48%). Stratifying by age, 182 donors (51.12%) were up to 45 years and 174 donors (48.88%) were older than 45 years. Out of 31 IgG-positive donors, three belonged to the first group (9.68%) and 28 (90.32%) to the latter group. Our analysis shows a trend that HEV-specific antibody positivity appears to be increased in male donors; however, statistical significance was not obtained ($P = 0.2678$; [−0.0276-0.0928]). Nonetheless, there is a strong association between IgG positivity and donor age ($P < 0.0001$; [0.0910-0.2082]; Figure 2). An identical trend was seen with HEV RNA-positive donors. From the 7 RNA-positive donors, five are male and two are female. Moreover, five donors are older than 45 years and two are younger (data not shown).

3.3 | Serological follow-up study

In a next step, donations from three additional time points (T_{-1}, T_1 and T_2; relative to T_0, the time point of initial screening) were analysed for all RNA-confirmed samples. This additional screening will allow us to evaluate the course of antibody appearance and disappearance. In addition, we also performed a similar kinetic analysis on the RNA-unconfirmed samples in the hope that serology could indicate those samples being potentially responsible for a positive HEV RNA result on pool level. All RNA-confirmed samples were IgM and IgG negative at T_{-1} (Table 2). Sample 5642_1 remained IgM and

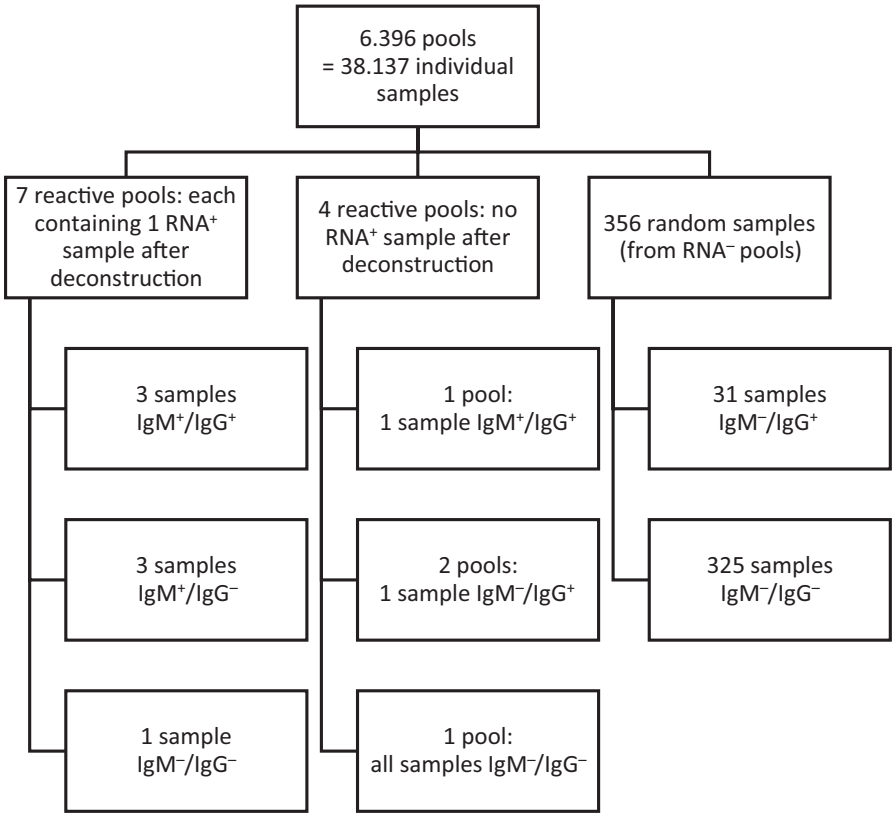

FIGURE 1 Serological analysis of HEV RNA reactive pools and a selection of 356 random samples that were HEV RNA negative when tested in pool format

TABLE 2 Serological analysis of prior and follow-up samples of RNA-confirmed and RNA-unconfirmed pools

	T_{-1}			T_0			T_1			T_2		
Sample ID	Time point[a]	IgM	IgG	Date[b]	IgM	IgG	Time point[a]	IgM	IgG	Time point[a]	IgM	IgG
RNA-confirmed samples												
6013_3	NA	NA	NA	08/2015	1, 25	6, 69	+5 mo	ND	20, 85	+23 mo	ND	18, 72
5642_1	−5 mo	ND	ND	08/2015	ND	ND	+13 mo	ND	17, 07	+16 mo	ND	18, 25
5397_2	−14 d	ND	ND	08/2015	1, 64	ND	+14 d	3, 30	5, 82	+28 d	2, 86	16, 73
2645_3	−6 mo	ND	ND	07/2015	4, 50	16, 03	+6 mo	ND	15, 37	+18 mo	ND	11, 32
5740_4	−12 d	ND	ND	08/2015	4, 79	17, 76	+15 d	2, 06	18, 52	+34 d	ND	20, 57
401_2	−14 d	ND	ND	06/2015	10, 76	ND	+14 d	ND	ND	+28 d	8, 16	17, 04
1096_3	−3 mo	ND	ND	06/2015	1, 25	ND	+5 mo	ND	16, 52	+8 mo	ND	17, 38
RNA-unconfirmed samples												
6417_5	−14 d	ND	2,63	08/2015	ND	2, 26	+14 d	ND	2,45	+56 d	ND	2, 41
2595_1	−3 mo	ND	ND	07/2015	ND	ND	+3 mo	ND	18, 17	+6 mo	ND	18, 97
4395_6	−2 mo	ND	ND	08/2015	ND	5, 93	+6 mo	ND	16, 94	+9 mo	ND	17, 25
6228_3	−3 mo	ND	ND	08/2015	2,20	17, 04	+5 mo	ND	19, 37	+8 mo	ND	19, 41

Note: T_0: time point of initial blood collection for HEV RNA screening.
Data are presented as optical density/cut-off (OD/CO) ratio.
Abbreviations: NA, not available; ND, not detected.
[a]Time point of prior and subsequent blood donations relative to T_0.
[b]Date (mo/y) of initial blood donation for HEV RNA screening.

IgG negative at T_0, but seroconversion was observed at T_1. The other RNA-confirmed samples seroconverted at T_0. Serological results of the RNA-unconfirmed samples reaffirmed that each pool contained one sample with positive serology. All other samples from RNA-unconfirmed pools were IgM and IgG negative at all time points investigated (data not shown). In one pool (6228), an IgM/IgG-double positive sample was present at T_0. At time point T_{-1}, this sample was seronegative, while follow-up samples only reacted positive for HEV IgG. In one RNA-unconfirmed pool (2595), we identified a donor (2595_1) who was seronegative at T_{-1} and T_0, but who seroconverted 3 months later. In pool 4395, one sample (4395_6) showed seroconversion at T_0 after being IgM and IgG negative at T_{-1}. Finally, we identified one individual in pool 6417 (6417_5) who was HEV IgG positive at all time points tested. Overall, one seropositive donor has been detected in all four RNA-unconfirmed pools and seroconversion is observed in three out of four individuals (Table 2).

4 | DISCUSSION

Transfusion-transmitted infectious diseases (TTIDs) remain a major subject of interest for the safety of blood donations.

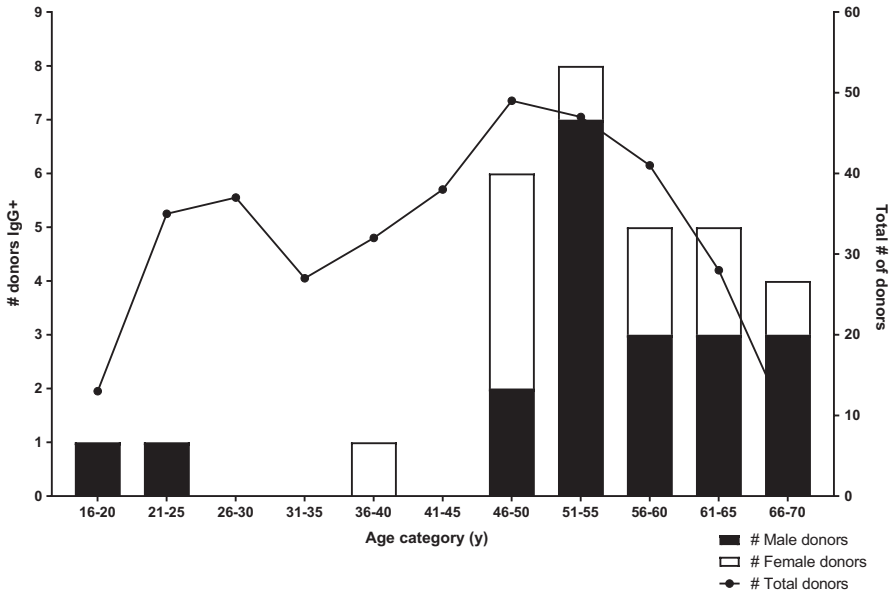

FIGURE 2 Age and gender distribution of IgG prevalence in the 356 randomly selected samples. The cumulative number of male (black bar) and female (white bar) donors being positive for anti-HEV IgG is displayed on the left axis. The total number of donors (solid line) is displayed on the right axis

Seemingly healthy, asymptomatic blood donors may transmit a whole range of blood-borne infectious agents through transfusion. In this paper, we assess the HEV prevalence in Flemish blood donors. The 38 137 analysed blood donations were first pooled per 6. Of the total amount of 6396 pools, 11 reacted positive. Nevertheless, only seven pools could be confirmed in a second individual PCR screening, where each pool contained only one HEV RNA-positive donation. Based on these results, we can conclude that 0.018% or 1:5448 donations are HEV RNA positive. All RNA positive donors were evenly distributed geographically. HEV RNA has been determined in blood donations in various other European countries, and rates of HEV positivity range from 1:726 (the Netherlands) to 1:3333 (Spain).[5,6] The most comprehensive study on transfusion-transmitted HEV was conducted in the UK. A screening of 225000 blood donations showed a HEV RNA prevalence of 1:2848. A follow-up study revealed a transmission rate of 42%.[7] Transmission through transfusion of blood has also been reported in other European countries such as Germany, Spain and France, but also in Canada and Japan.[2]

All individual samples from the 11 pools that tested positive during the first HEV RNA screening were screened for the presence of anti-HEV antibodies. In addition, to evaluate the sequence of antibody appearance, donations from three additional time points were analysed. All RNA-confirmed samples, apart from donor 401_2, showed a normal serological course. It remains unclear why donor 401_2 showed an intermittent IgM profile. IgM became undetectable 2 weeks after its initial observation, to become detectable again after 28 days. Reanalysis of the samples confirmed the above-mentioned results. Among the samples of RNA-unconfirmed pools, sample 6417_5 was IgG positive during all time points. This indicates an HEV infection that initiated prior to time point T_{-1}. Should this concern a recent infection, the T_0 sample might have had a borderline RNA level, thereby potentially explaining why the pool tested positive. In the remaining three RNA-unconfirmed pools, a sample was found that showed seroconversion, indicating that this sample most likely was responsible for the positive result during our initial pool level screening. If the HEV RNA-positive signal of the three seroconverted unconfirmed pools was indeed the consequence of borderline positive individual samples, the HEV RNA prevalence in our blood donor cohort would amount to 0.026% or 1:3814 donors.

To determine the anti-HEV IgG prevalence rate in Flemish blood donors, the serology screening was extended by 356 additional samples randomly selected from pools that were RNA negative during the first PCR screening. Antibody screening revealed 31 IgG-positive samples (IgM^-/IgG^+). Based on these results, we can conclude that the anti-HEV IgG prevalence rate is 8.71% (31/356). In 2012, a study was performed at the Ghent University Hospital (Belgium) to estimate the HEV prevalence in Belgium. A total of one hundred patients presenting at the gynaecological (mainly at the fertility centre) or orthopaedic clinics were randomly selected to be tested for the presence of HEV-specific IgG antibodies. The seroprevalence was found to be 14%.[8] However, compared to our study, a different ELISA assay was used and the tested population was less extended. When looking at seroprevalence studies of blood donations in other countries, a wide variation can be found with the lowest seroprevalence in Southwest Switzerland (4.2%) and the highest in Southwestern France (52.2%).[9] Nevertheless, different studies in one country performed almost at the same time revealed a wide variation in seroprevalence, which makes it challenging to estimate the true rate. When looking closer at studies using the same immunoassay as the one used in our study, considerable variability remains.[10-13]

To reveal possible epidemiological trends, age and gender data from the random samples have been compared. Anti-HEV IgG antibodies were detected principally in men older than 45 years. Nevertheless, the difference in gender was not statistically significant. However, several studies report that symptomatic hepatitis E patients are more likely to be male, suggesting that men, compared to women, are at higher risk of developing disease following exposure.[14-17]

The relevance of transfusion-transmitted HEV infections has been discussed by several European committees.[18] Taking the increase of HEV prevalence in the Western world into account, several countries already decided to implement a screening strategy.[5] Universal screening is already implemented in the Netherlands, Ireland and the UK.[5,19,20] In France and Germany, testing is currently limited to respectively a part of the plasma production for use in patients at high risk and all of the blood donations in certain blood centres. In both countries, a universal screening of blood donations is still under consideration.[5,21] In Belgium, blood donations are currently not screened for the presence of HEV. Based on our results, the necessity for screening Flemish blood donations can be debated. Cost-effectiveness studies have not yet been performed, but can be of importance for stakeholders to make a decision.[22]

During the window period, pathogens are not detected by donor screening strategies. To overcome this, pathogen inactivation (PI) technologies are being used to treat blood products.[23] There are several PI technologies available for the treatment of plasma and platelet concentrates, but PI methods for the treatment of RBC units are still in development and have not received market authorization yet.[23] Plasma units can be treated by Intercept (amotosalen), Mirasol (riboflavin), solvent/detergent (SD) and methylene blue. Platelet concentrates are treated with Intercept (amotosalen) or Mirasol (riboflavin). All PI methods are based on the principle of impairing the target pathogen's ability to replicate.[23] However, two cases of HEV transmission after transfusion of two Intercept-treated plasma units have been reported.[24] In contrast to Intercept and SD treatment, Mirasol was proven to inactivate greater than 2 to 3 logs of live HEV in platelet samples.[25] Data on methylene blue treatment towards HEV inactivation have not been reported.

To further reduce the risk of HEV infection in a blood donation recipient, it is important to keep the plasma volume of the donation as low as possible. In platelet concentrates, plasma can be partially replaced by platelet additive solutions (PASs) to reduce this residual plasma volume. In addition, red blood cells are manufactured by removal of plasma and storage in an additive solution and are not considered a high-plasma-volume component.[26] Limiting the residual

plasma volume in both platelet and red blood cell concentrates will further reduce the risk of HEV transmission.

In conclusion, our study shows that at least 1:5448 of blood donations at the Red Cross Flanders are HEV RNA positive. Upon transfusion, these donations may pose a major risk especially to immunocompromised patients or patients with pre-existing liver disease. Serological analysis showed that the anti-HEV IgG prevalence in Flemish blood donors is 8.71%. Our data provide a basis for discussion regarding the blood donation screening strategy in Belgium in terms of transfusion safety.

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CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

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